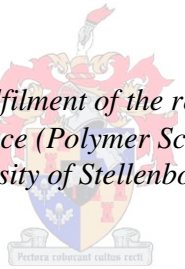
Two-dimensional chromatographic characterisation of PS-b-PEO copolymers at the critical conditions of their corresponding homopolymers

by

Monika Elvira Grabowsky



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Supervisor: Prof. Harald Pasch
Faculty of Science
Department of Chemistry and Polymer Science

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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Abstract

Block copolymers are very interesting materials but they are quite complex. During polymer synthesis only a certain amount of control can be enforced. As copolymers are made up of two or more different homopolymer segments, and therefore have different end group possibilities, varying block lengths and block sequences, they have complex structures and are therefore difficult to analyse.

Different techniques exist by which polymers can be analysed to determine the aforementioned distributions. In order to achieve a complete characterisation of a polymer structure, it is best to first use a separation technique to fractionate the polymer into more homogeneous fractions, and then use identification techniques to analyse these fractions.

Polystyrene-block-poly(ethylene oxide) (PS-b-PEO) copolymers were investigated using liquid chromatography at the critical conditions (LCCC) of the copolymers' corresponding homopolymers, two-dimensional liquid chromatography (2D-LC) and FTIR. The block copolymers were analysed using the established LCCC of PS but it was found that even though separation of PS homopolymer and copolymer was obtained, PS blocks of the copolymers contributed to some extent to the retention of the PEO blocks.

Some of the block copolymer samples were fractionated at the established critical conditions of PS. These fractions were qualitatively and quantitatively analysed using FTIR spectroscopy. The settings for the 2D-LC analysis were established, using LCCC of PS as the first dimension and as the second dimension SEC, using DMF as eluent. DMF was a suitable solvent to be used for the second dimension because PS, PEO and PS-b-PEO exhibited good solubility in this solvent. THF did not dissolve the block copolymers completely.

The same solvent system as used for LCCC of PS was used for LCCC of PEO, but the critical conditions correspond to a different solvent composition. The block copolymers were analysed using the established LCCC of PEO but it was found that even though separation of PEO homopolymer and copolymer was obtained, the PEO blocks of the copolymers contributed to some extent to the retention of the PS blocks. Some of the block copolymer samples were fractionated at the established critical conditions of PEO. These fractions were qualitatively and quantitatively analysed using FTIR spectroscopy. The settings for the 2D-LC analysis were established, using LCCC of PEO as the first dimension and as the second dimension SEC using DMF as eluent was used. Lastly, qualitative and quantitative analyses of the block copolymers were carried out using FTIR spectroscopy.

Opsomming

Alhoewel blokkopolimere baie interessante verbindings is, is hulle redelik ingewikkeld. Gedurende die kopolimerisasiereaksie kan daar net 'n sekere mate van kontrole behaal word. Aangesien kopolimere uit twee of meer homopolimeersegmente, met verskillende end-groep moontlikhede, bloklengtes en blokvolgordes bestaan, is dit baie moeilik om hierdie verbindings te analiseer.

Verskillende tegnieke kan gebruik word vir die analise van polimere en die bepaling van bogenoemde verspreidings. Ten einde 'n polimeerstruktuur volledig te karakteriseer is die beste manier om eers 'n skeidingstegniek te gebruik om die polimeer in meer homogene fraksies te fraksioneer en dan daarna hierdie fraksies te analiseer.

Polistireen-blok-poli(etileenoksied) (PS-b-PEO) kopolimere is ondersoek deur gebruik te maak van vloeistofchromatografie by kritiese kondisies (LCCC) van die kopolimeer se ooreenkomstige homopolimere; twee-dimensionele vloeistofchromatografie (2D-LC) en FTIR. Die blokkopolimere is gekarakteriseer deur gebuik te maak van bevestigde LCCC van PS. Daar is egter gevind dat alhoewel skeiding van die PS homopolimeer en die kopolimeer behaal is, PS blokke van die kopolimere in 'n mate bygedra het tot die retensie van die PEO blokke.

Sommige van die blok-kopolimeermonsters is gefraksioneer by die bepaalde kritiese kondisies van PS. Hierdie fraksies is kwalitatief en kwantitatief geanaliseer deur gebruik te maak van FTIR spektroskopie. Die stellings vir die 2D-LC analise is bepaal deur gebruik te maak van LCCC van PS as die eerste dimensie en SEC as die tweede dimensie, met DMF as elueermiddel. DMF was 'n geskikte oplosmiddel vir die tweede dimensie aangesien PS, PEO en PS-b-PEO goed oplosbaar is daarin. Die blokkopolimere was nie volledig oplosbaar in THF nie.

Dieselfde oplosmiddelsisteem soos gebruik vir die LCCC van PS is gebruik vir die LCCC van PEO, maar die kritiese kondisies stem ooreen met 'n ander oplosmiddelsamestelling. Die blokkopolimere is geanaliseer deur gebruik te maak van die bevestigde LCCC van PEO, maar daar is bevind dat alhoewel skeiding van die PEO homopolimeer en kopolimeer behaal is, die PEO blokke van die kopolimere in 'n mate bygedra het tot die retensie van die PS blokke. Sommige van die blokkopolimeermonsters is gefraksioneer by die bevestigde kritiese kondisies van PEO. Hierdie fraksies is kwalitatief en kwantitatief geanaliseer deur gebruik te maak van FTIR spektroskopie. Die stellings vir die 2D-LC analise is bepaal deur gebruik te maak van

LCCC van PEO as die eerste dimensie en SEC as die tweede dimensie, met DMF as elueermiddel. Laastens is kwalitatiewe en kwanitatiewe analises van die blokkopolimere m.b.v. FTIR spektroskopie uitgevoer.

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All honour to my Abba Father.
Thank you for always picking me up and giving me hope (Isaiah 35:3-7) again for the future that you have promised me - and for every opportunity you gave me to grow.

Table of Contents

List of abbreviations

2D-LC	-	two-dimensional liquid chromatography
C18	-	octadecyl
CCD	-	chemical composition distribution
CRYSTAF	-	crystallisation analysis fractionation
DMF	-	N,N-dimethylformamide
ELSD	-	evaporative light scattering detector
F#	-	fraction e.g. one
FTD	-	functionality type distribution
FTIR	-	Fourier-transform infrared spectroscopy
V_h	-	hydrodynamic volume
HPLC	-	high-performance liquid chromatography
LAC	-	liquid adsorption chromatography
LC	-	liquid chromatography
LC-CAP	-	LC at the critical adsorption point
LCCC	-	liquid chromatography at critical conditions
comp-2D-LC	-	comprehensive two-dimensional liquid chromatography
linear-2D-LC	-	linear (“heart-cutting”) two-dimensional liquid chromatography
LC-PEAT	-	LC at the point of exclusion-adsorption transition
M	-	molecular weight
M_n	-	number average molecular weight
M_p	-	molecular weight at the peak maximum
MS	-	mass spectroscopy
M_w	-	weight average molecular weight
MWD	-	molecular weight distribution
NMR	-	nuclear magnetic resonance spectroscopy
NP	-	normal phase
PEO	-	poly(ethylene oxide)
PS	-	polystyrene
PS-b-PEO	-	polystyrene-block-poly(ethylene oxide) copolymer
RI	-	refractive index
RP	-	reversed phase
SEC	-	size exclusion chromatography
SLM	-	standard litres per minute
T	-	temperature
THF	-	tetrahydrofuran
TREF	-	temperature rising elution fractionation
UV	-	ultraviolet

V_e	-	elution volume
V_0	-	void volume
LCCC x SEC	-	LCCC (first dimension analytical method) coupled with SEC (second dimension analytical method)
gradient-HPLC x SEC	-	gradient HPLC (first dimension analytical method) coupled with SEC (second dimension analytical method)

List of symbols

ΔH	-	change in enthalpy
ΔS	-	change in entropy

List of Figures

List of Tables

Chapter 1

Introduction and objectives

1.1. Introduction

Block copolymers are interesting materials. Copolymerising two or more monomer types results in copolymers that have a combination of different properties. There are different types of copolymers, such as random, statistical, block and grafted. For the random type the monomers are copolymerised in a random way, while for the statistical type the different monomers are added in specific orders and in specific quantities so that in the end a copolymer results where the sequence of each type of monomer (monomer A and monomer B) increases, e.g. A-B-AA-BB-AAA-BBB-AAAA-BBBB. A block and grafted copolymer is made up, for example, of two different homopolymers, where for the block copolymers the two different monomers a polymerised sequentially so that homopolymer blocks are formed that are covalently bound to each other. In graft copolymers the backbone is one type of polymer from which the other type of homopolymer is grafted.

All these types of copolymers have their advantages and applications. For example, a hydrophobic-hydrophilic block copolymer such as polystyrene-block-poly(ethylene oxide) copolymer (PS-b-PEO) is often used for solubilisation, emulsification, stabilisation, as surfactants and as detergents, in drug delivery, templating[1], and also for removal/recovery of organic/inorganic compounds from contaminated waters[2], to name a few.

For the synthesis of copolymers only a certain amount of control can be exerted. Therefore the end product of a copolymerisation is often a mixture of copolymer and its corresponding homopolymers. The properties of such an end product vary, depending on factors such as the amount, chemical composition and polydispersitiy of the copolymer, the amount of the homopolymer, etc. When more homopolymer is present the properties correspond more to a blend rather than a copolymer. Therefore these products need to be analysed in order to determine, for example, the amount of homopolymer present after the completion of copolymerisation.

To analyse such a complex product, it must be first separated, otherwise one will not have a clear picture of the end product. A suitable separation method would be high performance

liquid chromatography (HPLC). HPLC can be used in different separation modes, such as size exclusion chromatography (SEC), liquid adsorption chromatography (LAC), and liquid chromatography at critical conditions (LCCC). Each one of them can be applied to achieve a certain separation. For the SEC mode, the molecules elute according to the size of the polymer chains and for LAC the molecules elute according to, for example, functional end groups. For the LCCC mode, at the critical conditions of a specific part of a copolymer all the molecules (of the same chemical composition) elute at the same elution volumes independent of molecular weight. While operating at the critical conditions of one part of the copolymer (e.g. polystyrene (PS)) the other part will either elute in the SEC or the LAC mode depending on factors such as the polarity of the stationary phase, polarity of the polymer, the operating temperature and the solvent composition used.

For even more information about the molecular heterogeneity two-dimensional liquid chromatography (2D-LC) is a useful analysis technique. For this technique, two analytical methods are combined to give information on different aspects of molecular heterogeneity in one experiment. An example would be the use of a method in the first dimension that separates according to chemical composition and another method that separates according to size in the second dimension. In the first dimension the sample will be separated into fractions that are chemically homogeneous[3]. These fractions are than transferred into the second dimension where they undergo separation according to size. The information obtained after performing such a separation is the molecular weight of each homogenous fraction.

In this study, PS-b-PEO will be investigated. LCCC of PS and PEO will be established while the other non-critical part of the copolymer will elute in the SEC mode. Furthermore, 2D-LC, where LCCC in the first dimension will be coupled to SEC in the second dimension, will be used to obtain information about the molecular weights of possible homopolymers as by-products. FTIR spectroscopy will be used to obtain qualitative and quantitative information about the chemical composition of the original samples and their fractions.

1.2. Objectives

The main objectives were: 1. to establish critical conditions of PS, 2. to establish critical conditions of PEO, 3. to analyse the molecular heterogeneity of a series of PS-b-PEO block copolymers using these critical conditions. These were divided into separate tasks.

1. Establishing Critical conditions of PS
    - Find suitable solvents and solvent combinations to dissolve PS, PEO and PS-b-PEO
    - Analyse, qualitatively and quantitatively, the block copolymers with FTIR.
    - Establish critical conditions of PS for a given solvent combination by varying the composition of this solvent combination.
    - Analyse the block copolymers with the established critical conditions of PS
    - Fractionation of block copolymer samples where necessary.
        - Qualitative and quantitative analyses of the fractions with FTIR
    - Establish 2D-LC settings, using critical conditions of PS as the first dimension and SEC as the second dimension.
        - Finding a suitable eluent for SEC as the second dimension.
2. Establishing critical conditions of PEO
    - Find suitable solvents and solvent combinations to dissolve PS, PEO and PS-b-PEO
    - Analyse, qualitatively and quantitatively, the block copolymers with FTIR.
    - Establish critical conditions of PEO for a given solvent combination by varying the composition of this solvent combination
    - Analyse the block copolymers with the established critical conditions of PEO
    - Fractionation of block copolymer samples where necessary.
        - Qualitative and quantitative analyses of the fractions with FTIR
    - Establish 2D-LC settings, using critical conditions of PEO as the first dimension and SEC as the second dimension.
        - Finding a suitable eluent for SEC as the second dimension.

1.3. References

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2. Hadjichristidis, N.; Pispas, S.; Floudas, G. A., *Block Copolymers: Synthetic Strategies, Physical Properties, and Applications*. John Wiley & Sons: Hoboken, New Jersey, 2003.

3. Pasch, H. *Macromolecular Symposia* **2001,** 174, (1), 403-412.

Chapter 2

Literature Review

2.1. Block copolymers and their synthesis via living anionic copolymerisation

Copolymers are made up of two or more different types of monomers that are chemically bonded. Copolymers can be subdivided into graft, star, statistical or random, and block copolymers. An advantage of copolymers, e.g., block copolymers, is that some of the properties of the individual homopolymers may be improved. Block copolymers, specifically, can be diblock, triblock or even multiblock copolymers. As they are made up of two or more different homopolymer segments, and therefore have different end group possibilities, varying block lengths and block sequences, they have complex structures and are therefore difficult to analyse.

Block copolymers have many different applications; they can be used for solubilisation, emulsification, stabilisation, as surfactants and as detergents, in drug delivery, templating[1], and also for removal/recovery of organic/inorganic compounds from contaminated waters[2], to mention a few.

Various synthetic methods can be used for the synthesis of block copolymers, for example radical and ionic polymerisation. Anionic polymerisation is most often used as it gives a highly controlled end product, with control over the molecular weight (over all and for the blocks), end groups, composition and chain architectures. With free radical polymerisation, coupling or radical transfer reactions are common side reactions which lead to a lesser controlled end product with different side products. Details of the synthesis of block copolymers are well described in references[2-5]. The focus of this study was on the analysis of diblock copolymers, prepared via living anionic polymerisation.

Living anionic polymerisation involves two main steps: chain initiation and chain propagation (and no formal termination reaction). Chain termination reactions occur if a termination agent is added or some impurities are present in the reaction mixture. The absence of a formal termination step gives the living anionic polymerisation an advantage over other polymerisation techniques, resulting in good control over the end product. Another advantage

is that the polymerisation continues until all monomer is consumed, but continues again as soon as more monomer (the same or different) is added. The molecular weight of the polymer i.e. the individual blocks of a copolymer can be controlled by adding a predetermined amount of monomer. If a different monomer type is added then a diblock copolymer is formed.

Poly(styrene-block-ethylene oxide) (PS-b-PEO) was the polymer used in this study and therefore it will be used as an example for the explanation of the living anionic polymerisation technique. A similar reaction mechanism is generally applicable to other types of block copolymers. A general summary of living anionic polymerisation can be found in the papers of Webster[5] and Quirk et al.[4]. The preparation of (PS-b-PEO) is carried out in tetrahydrofuran (THF) solution at -78 °C. The polymerization is initiated by an initiator such as cumyl potassium. The composition of PS-b-PEO is controlled by starting the polymerisation with one type of monomer, e.g., styrene, and polymerisation continues until all the styrene monomer is consumed. This is then followed by the addition of the next monomer, ethylene oxide. For example, a specific amount of purified ethylene oxide is added while the reaction solution is kept between room temperature and 40 °C. The ethylene oxide monomer adds on to the already formed but still living polystyrene (PS) block until all of it is consumed. At this point, a termination agent is usually added, followed by isolation of the polymer. The isolation step involves precipitation in a nonsolvent[2]. The end product is a diblock copolymer consisting of PS and polyethylene oxide (PEO) blocks in addition to some of the homopolymer of either or both PS and PEO. The presence of the homopolymer is usually the result of a termination reaction, due to impurities incorporated into the system or added with the second monomer. There is also a slight possibility that coupling reactions may occur.

2.2. Analysis of polymer chemical structure

During polymer synthesis only a certain amount of control can be enforced. The end product could have distributions in, for example, chain lengths, end group functionality and the architecture of the chains. Different techniques exist with which polymers can be analysed in order to determine the different aforementioned distributions. In order to achieve a complete characterisation of a polymer structure, it is best to first use a separation technique to

fractionate the polymer into more homogeneous fractions and then use identification techniques to analyse these fractions. Separation techniques are mostly liquid chromatography techniques such as high-performance liquid chromatography (HPLC). Depending on the separation mechanism HPLC methods can be divided into size-exclusion chromatography (SEC), liquid adsorption chromatography (LAC) and liquid chromatography at the critical point of adsorption (LCCC). Identification methods often involve the use of different types of detectors, such as the ultraviolet (UV) and refractive index (RI) detectors, and spectroscopy techniques such as Fourier transform-infrared spectroscopy (FTIR), mass spectroscopy (MS) and nuclear magnetic resonance (NMR). In this study HPLC with evaporative light scattering (ELSD) and UV detectors, and FTIR as identification method, were used and are briefly discussed in **Sections 2.2.3**.

2.2.1. High-performance liquid chromatography

In HPLC a porous column packing is usually used as the stationary phase due to its high surface area. HPLC can be subdivided into three main modes; SEC, liquid chromatography at critical conditions (LCCC) and liquid adsorption chromatography (LAC).

For the ideal SEC condition, the entropy (ΔS) < 0 and the enthalpy (ΔH) = 0 thus is the Gibbs free energy (ΔG) < 0. The separation is based on the hydrodynamic volume (the size that the molecule adopts in solution, V_h) of the polymer in solution where the longer chains usually have a larger V_h than the shorter ones. The ideal LAC mode is only governed by ΔH and $\Delta S = 0$, but due to the use of porous packed columns both ΔS and ΔH contribute to the solute retention[6]. The LAC separation is based on interactions of the polymers with the stationary phase. These selective interactions can be either adsorption due to polarity, hydrophobicity, charge transfer etc. For the LCCC mode, at the critical condition of a specific part of a copolymer or of a polymer with functional end groups, all the molecules (of the same chemical composition) elute at the same elution volume independent of molecular weight. At the ideal LCCC, $\Delta G = 0$, because the $T\Delta S$ and the ΔH counterbalance each other. Therefore LCCC is a good method to establish separation according to chemical composition irrespective of molecular weight.

The experimental conditions for the different separation modes and the type of polymer to be analysed determines in which mode the polymer chains will elute. The solvent ideally used to obtain the SEC mode needs to suppress any enthalpic interaction between the stationary phase and the polymer sample. The more enthalpic interaction there is allowed to be established between the stationary phase and the polymer the more the separation mode changes from SEC to LAC. In between these two modes lies the LCCC mode where the polymer sample in the ideal conditions does not experience any retention. This, however, is only true for linear homopolymers without interacting end groups. Thus, there is generally some retention experienced, due to some type of interaction between the polymer, column and solvent used. An example of a molecular weight versus elution volume plot can be seen in **Figure 2.1**. More details about the general theory of LC of polymers can be found in the reviews of Philipsen[7] and Berek[8]. The different LC modes will be discussed in the following section (**Sections 2.2.1.1–2.2.1.3**).

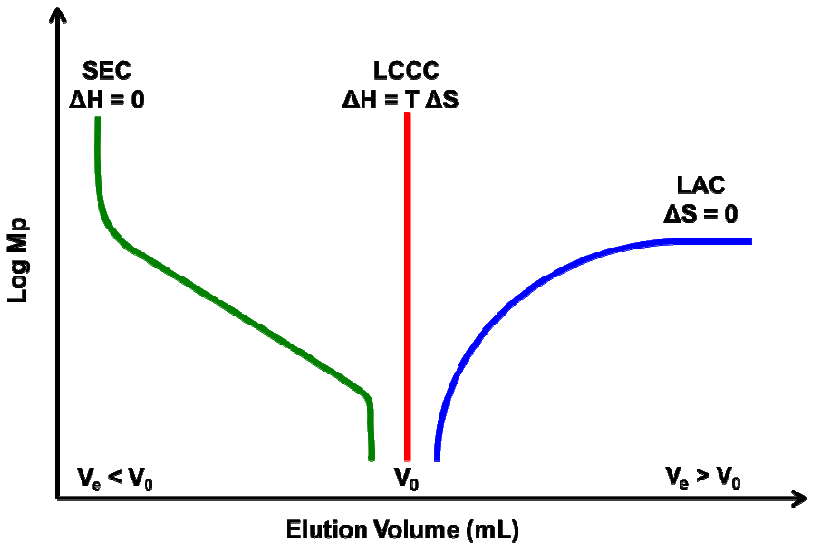

Figure 2.1: A representation of molecular weight versus retention volume in the three different modes possible in LC.

2.2.1.1. Size-exclusion chromatography

SEC columns are packed with porous beads. Columns can be chosen with specific pore sizes, for example, 100, 300, 1000 Å. Each type can be used for specific polymer molecular weight

ranges. For example, for the lower molecular weight range the 100 Å pore size is suitable. Above that (Mp above 35000 g/mol) the chains are completely excluded from the pores of the stationary phase and will elute with the void volume (V_0); the void volume also called the dead volume is the total volume of the mobile phase in the chromatographic column. The shorter chains are able to enter the pores, having the longest path length through the column while the longer chains are generally too large so they enter only few or none of the pores and have the shortest path length through the column. The polymer chains are thus sorted by size and the order of elution will be the longest chains eluting first and the shortest last. Ideally, as already mentioned, in SEC the enthalpy contribution should be zero; however, in practice this is mostly not the case. The aim is to make it as small as possible by choosing a thermodynamically good solvent and a column packing which is inert to selective (adsorptive) interactions.

Advantages of SEC over other methods for the characterisation of the molecular weight of polydispersed polymers are quick analysis, less effort for preparing the samples for the analysis and little amount of sample is required[9]. The main disadvantage is that it needs to be calibrated with standards which should be either the same type of polymer as the sample of interest or a closely related polymer type. In the latter case, only the molecular weight relative to that standard is determined and thus the value found is not absolute. Another disadvantage is that there is an upper and lower limit of polymer chain size which can be used for specific columns with a given pore size. Thus, if needed, combinations of different pore sizes (e.g.100, 300, 1000 Å) should be used. Furthermore, SEC works very well for linear and chemically homogenous types of polymers but not so well for complex polymers such as mixtures of homopolymers, heterogeneous copolymers, polymers with different molecular architectures as it cannot separate polymer species of different nature with similar hydrodynamic volumes. Within a given complex polymer, different parts or chains have a different degree of interaction with the solvent and therefore might take up the same size in solution, in other words the same hydrodynamic volume which would then result in co-elution of these molecules as it is illustrated in **Figure 2.2**.

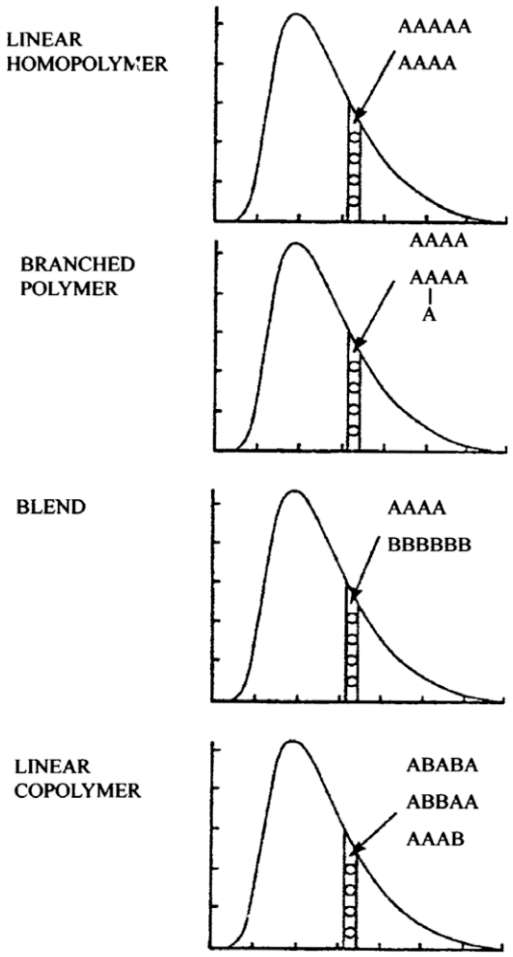

Figure 2.2: Schematic representation of possible co-elution of polymer species of different nature with similar hydrodynamic volumes that may affect the SEC separation of polymers[9].

2.2.1.2. Liquid adsorption chromatography

As mentioned previously, LAC is a method where the polymer molecules are permitted to interact with the stationary phase. It is mainly controlled by enthalpic interactions. The degree of interaction between the polymer molecules and the stationary phase is governed by the strength and polarity of the mobile phase as well as the type of stationary phase (its polarity and its pore size distribution). In other words for a polar polymer a polar stationary phase is used such as a silica stationary phase while for a non-polar polymer a non-polar stationary phase is used (e.g. C18 modified stationary phase). The enthalpic interactions mentioned above are not only affected by the chain end groups or the polymer chain itself but also by the overall polarity of the molecules[10]. An increase in length of the non-polar end groups will

cause the total polarity of the molecule to decrease and thus the polar interactions with the polar stationary phase will decrease. Therefore molecules with longer non-polar end groups will elute first, retention increases exponentially with the number of adsorbing groups in a molecule[11].

LAC analysis mainly depends on the interaction enthalpy and temperature shows a strong influence on this mode. The solute's retention generally decreases with the increase in temperature[6]. An increase of temperature has an effect similar to increasing the solvent strength by increasing the amount of the strong solvent in the composition of the mobile phase, depending on type of polymer analysed and stationary phase used.

A disadvantage of the LAC mode is that it is restricted to lower molecular weight polymers because the larger molecules will irreversibly adsorb onto the column. Thus, this mode is often only applied for end group analysis and low molecular weight polymers, when isocratic elution is applied. When using a gradient elution system (the solvent composition of the mobile phase is changed gradually during the analysis time) the much higher molecular weight samples can be analysed with this mode.

2.2.1.3. Liquid chromatography at the critical point of adsorption

LCCC is a very helpful method to analyse complex polymers such as diblock copolymers where the blocks are synthesised of monomer A and B, respectively. The reason why this method is a good one to analyse complex polymers such as block copolymers is that one part, the one not to be analysed in the moment, can be made "chromatographically invisible" so that the polymer part of interest such as one specific block of a block copolymer can be separated (analysed) irrespective of the other part. The term "chromatographic invisibility" just indicates that this part of the macromolecule does not contribute to retention. In order to determine the critical point for one of the blocks, its corresponding homopolymer calibration standards will be used. The process where the critical point for a specific part of copolymer is determined can also be described as making that part chromatographically invisible while the other part will be "visible". At that point there is compensation between the enthalpy and the entropy

terms and thus the free energy change will be zero. Therefore, the calibration standards will co-elute with the solvent peak because they do not experience any retention within or on the stationary phase. In the case of a block copolymer, the invisible block will therefore not contribute to the retention of the visible part; hence the retention effect of the copolymer will be only due to the visible part of it. The reason that the invisible block will not contribute to the other blocks retention is that it is independent of the molecular weight in those critical conditions and is therefore only governed by chemical differences. Factors which need to be looked at when establishing the critical conditions for one of the parts of a copolymer is the chemical structure of that specific part, the nature of the stationary phase and appropriate solvent[12]. There are different methods of how to obtain a critical point, such as using specific solvent mixtures at a certain ratio composition, controlling and varying temperature or the pH when aqueous solvents are used[13]. The main attention when selecting the appropriate solvents for LCCC needs to be given to evaluate the polarities of both the stationary phase and mobile phase in comparison to the polarities of the monomer units[14].

There are two types of elution procedures among others which are often used for HPLC therefore can also be used for LCCC. The difference of these two groups lays in their polarity; where in the one the stationary phase is much more polar than the mobile phase and the other one it is the other way around. The first elution procedure is termed normal phase (NP) and the other one reverse phase (RP). Silica is often used for the NP and a C18 modified silica is used for the RP. Irrelevant if NP or RP is used, as the amount of the strong solvent ("strong solvent, which fully suppresses adsorption of a polymer on a given column packing at given temperature (and pressure)"[15]) increases in the mobile phase, for NP making mobile phase less polar than the stationary phase and more polar for RP, thus decreasing interactions between the polymer sample and the stationary phase, the more the sample will elute in the SEC mode. By decreasing the solvent strength (in other words, increasing the weak solvent, which promotes full adsorption of a polymer on a given column packing at given temperature (and pressure)"[15]), allowing the interaction between the sample and the stationary phase to be increased, the sample will elute more to LAC mode. The critical point lies in between those two modes; this corresponds to a specific combination of the strong and poor solvents. The

precise solvent ratio at which the critical point is found depends on a number of factors, e.g., the polymer to be analysed, the stationary phase used, at which temperature the analysis is done etc. This is the compensation point of ΔS and ΔH.

In LCCC analysis, when using NP silica columns it is advisable to find the critical point of the more polar component because then it can be expected that the other component will elute in the SEC mode[16]. When operating at the critical point for the less polar block, the more polar one will elute in the LAC mode; the disadvantage of operating in this way, as mentioned in the previous section of LAC, is that for the visible block there is an upper molecular weight limit where the molecules will adsorb irreversibly to the stationary phase. To overcome this limitation the separation process can be reversed by using a RP (e.g., C18) column. This entails that when using similar analysis condition as before the molecules will then elute in the SEC mode rather than LAC. The molecular weight dependencies of these two modes can be seen in the graph in **Figure 2.1.** An advantage of eluting the low molecular weight samples in the LAC mode is that it allows higher resolution separation compared to the SEC mode[6]. The reason for the latter is the lower band broadening in the LAC mode.

The reliability of the concept of chromatographic invisibility was investigated by different research groups and their conclusions are different. Pasch et al.[17,18] analysed polystyrene-block- poly(methyl methacrylate) (PS-b-PMMA) copolymers with LCCC method and came to the conclusion that the "invisibility" concept is reliable. They have established critical conditions for PMMA and analysed the block copolymer plus its corresponding PS precursors. It was observed that at the critical condition of PMMA the block copolymers behave like their corresponding PS precursors. Therefore it was concluded that the separation of the copolymer at the critical condition of PMMA is due to the PS block and that the block of PMMA does not contribute to the retention[17,18].

Falkenhagen and co-workers[19] synthesised poly(methyl methacrylate)-block-poly(tert-butyl methacrylate) (PMMA-b-PtBMA) copolymers where for one block the length is kept constant while for the other block the lengths were varied. The synthesis was repeated but in this case

the other block's lengths were kept constant while the former one is varied. Both these copolymer types were then analysed on a NP and a RP column respectively, so that the other constant length block component eluted in the SEC mode. It was found that the retention time was the same irrespective of the varied block length of the invisible block. Therefore, the retention time of the copolymers only depended on the other (visible) block component[19] and thus showing again the reliability of the analytical method of critical condition.

Philipsen et al. [20] and Lee et al. [21,22] on the other hand, found that the critical condition concept is not always very reliable. This is because the retention of polymers is quite sensitive to small differences in parameters, such as solvent compositions, stationary phases, and temperature to name a few. Philipsen et al.[20] found that if the solvent composition used to dissolve the sample and the mobile phase composition differ even slightly (difference as small as 1 vol.%) zone splitting might be caused. This problem is slightly more pronounced when very volatile solvents are used for the analysis. They also observed an increase in peak broadening when going from SEC to LCCC mode, especially for higher molecular weight polymers. The peak broadening is in general not very favourable. They conclude their study with the statement[20]: "In our opinion liquid chromatography under critical conditions is a feasible technique which can provide unique information on polymeric microstructures in special cases. Some 'critical' aspects, however, seem to have been underestimated until now. Further research can give more insight in possibilities and limitations of this useful technique."

Lee and co-workers[21,22] used a single solvent system where the temperature was adjusted in order to establish critical conditions. The reason for that was to make the critical condition more reproducible. In one case, similar to Falkenhagen et al.[19], they synthesised two series of polystyrene-block-polyisoprene (PS-b-PI) copolymers where for each series the length of one type of block is kept constant: a SI (styrene series) series with constant PS block length and an IS (isoprene series) series keeping the PI block constant[21]. In the other case PS-b-PI was also used but here they did not synthesise the polymer with controlled block lengths[22]. In both cases they have found that there is a dependency of the elution behaviour on the block length of the block at the critical point[21,22]. They observed that the retention of the visible block

elution in the SEC mode increased, and that the apparent molecular weight of the block decreases with the increase of the invisible block length[6]. It was found that a molecular weight difference, between both blocks, of a factor of two led to approximate 10% molecular weight error of the visible block.

There are various terms used for LCCC, such as LC at the point of exclusion-adsorption transition (LC-PEAT or LC-EATP), LC at the critical adsorption point (LC-CAP or LC-CPA). More details about the theory behind LCCC can be found in the works of Skvortsov and co-workers[13,23].

For a complete analysis of complex polymers with *n* independent properties there is a minimum of *n* independent characterization methods required[24]. For example, for a sample which has a distribution in chemical composition and a second distribution in molecular weight, two methods are needed to analyse both distributions. Two uncoupled methods can be used but the information obtained in total is often unclear and far from satisfactory thus when coupling the two methods much more information and better insight can be obtained. The analysis method where two methods are coupled is referred to as two-dimensional liquid chromatography (2D-LC).

2.2.2. Two-dimensional liquid chromatography

2.2.2.1. Introduction

2D-LC is an excellent method for the analysis of complex copolymers that have more than one distribution as described in **Section 2.2**. Information on different aspects of molecular heterogeneity can be obtained in one experiment. The combination of analytical methods for 2D-LC should be chosen in such a way that the second method is orthogonal to the first one[7]. In other words, the analytical methods that should be coupled should ideally be completely independent of each other. Each method should respond to only one specific molecular characteristic of the sample of interest, however, in practice this is rarely possible.

2.2.2.2. Analytical methods

Many analysis methods are influenced by more than one characteristic, as in the case of SEC[7]. SEC separation is based on V_h, as already mentioned, and V_h is not only influenced by the chain length but also by the chemical composition. Generally, the method with the highest selectivity for one specific characteristic and no (or hardly any) selectivity for any other characteristic of the sample to be analysed should be used in the first dimension[7]. A good choice for the first dimension is interaction chromatography (e.g. LAC or LCCC), because is the most adjustable one. Factors that can be adjusted to fine tune the separation according to chemical composition of the sample are, for example, the mobile phase, mobile phase composition, stationary phase and temperature[24]. Such fine tuning allows for a more homogeneous separation. Another reason for using interaction chromatography in the first dimension is that the sample load on the column can be much higher compared to SEC columns[24]. For the second dimension SEC is often chosen. SEC in the second dimension has the advantage that many different detectors can be used[7].

2.2.2.3. Off-line and on-line linear 2D-LC methods

A couple of years ago, before on-line 2D-LC was introduced, off-line 2D-LC was used. Fractions from the first separation method were collected i.e. with the help of a fraction collector and then re-injected into the second separation system i.e. manually or with the help of an auto-sampler. This however had some disadvantages such as contamination, loss or degradation of sample during solvent evaporation[25]. It was also a labour intense method and repeatability was a problem but it has the advantage that both dimensions can be run at their optimal flow rate and thus a good resolution for both dimensions can be obtained[11].

To overcome these disadvantages, on-line linear ("heart-cutting") two-dimensional liquid chromatography (linear-2D-LC) was used, where "heart-cuts" from the first dimension were collected in a storage loop which was then injected onto the second dimension column[25]. In other words, only some selected fractions and not the complete separated sample from first dimension were transferred into the second dimension to undergo separation. The latter is thus a drawback of this type of 2D-LC method. Thus making this technique only suitable for uses

in cases where only specific parts of the sample need to be analysed. Later comprehensive two-dimensional liquid chromatography (comp-2D-LC) was introduced which is also an on-line method but here the complete sample is analysed, and not only parts of it.

2.2.2.4. Comprehensive two-dimensional liquid chromatography (comp-2D-LC)

Comp-2D-LC has more advantages than linear-2D-LC. For example, with comp-2D-LC the complete analyte from the first dimension separation is introduced onto the second dimension analysis method. No intermediate re-concentration step is necessary thus the risk of sample contamination or oxidation is greatly reduced. Only a small quantity of the analyte is required to obtain maximum information, and a detailed quantitative interpretation of the results is possible[25].

2.2.2.5. Comprehensive two-dimensional liquid chromatography: setup

The two methods chosen to be used for the comp-2D-LC system are connected via an electrically triggered valve equipped with two sample loops[24]. Each loop, usually of 100 µL capacity, is filled with 100 µL fractions of the separated product from the first dimension. The first sample loop is filled. Then the valve is switched and that fraction is injected and separated in the second dimension. During the time where the first loop's fraction undergoes separation the second loop is filled. If the separation in the second dimension is done the valve is switched again and the fraction of the second loop is injected and separated while the first loop is filled again. This is repeated until the analysis is complete. The flow rates for these two methods used for 2D-LC need to be optimised in such a way (very low flow rate for the first dimension and a very high one for the second dimension) so that the time needed to fill one sample loop with one fraction (depending on the capacity of the sample loop) and the time needed for one fraction to undergo complete separation in the second dimension is the same.

The analysis time for the comp-2D-LC system with a flow rate of 0.025 mL/min in the first dimension and 1.5 mL/min in the second dimension is approximately 6 hours. There are theoretically two ways to reduce the analysis time. One is to increase the loop volume of the

switching system from 100 µL to 200 µL, but this would lead to a poor separation. The other is to increase the flow rate from 1.5 mL to 2.0 mL (or even higher) in the second dimension, but then it is advisable to use a high-speed SEC column to avoid high back pressure problems[10]. By using a high-speed SEC column the analysis time for the SEC dimension can be reduced by approximately a factor of about 10 without loss of resolution[24].More details about the experimental setup of 2D-LC separation can be found in the review of Kilz[24].

A significant point that needs to be taken into account when carrying out 2D-LC is the compatibility of the solvents of the different dimensions[7]. The mobile phases used for the different dimensions must be completely miscible. If the mobile phases are not completely miscible the separation of the second dimension is significantly influenced and the fraction might not be completely transferred into the second dimension. A good way to overcome this compatibility problem is by using the solvent of the second dimension as one of the solvents for the solvent composition of the first dimension[24] when using, for example, a comp-2D-LC where LCCC is used in the first dimension and SEC in the second dimension (LCCC x SEC).

2.2.2.6. Comprehensive two-dimensional liquid chromatography: advantages

As mentioned earlier, the advantage of 2D-LC is that much more information can be obtained compared to the "summed-up" information from the individual analytical methods used for the 2D-LC. An example of information that can be obtained for a block copolymer analysed by LCCC x SEC is the individual block lengths of the copolymer and also how much homopolymer was formed during the synthesis. When using, for example, only SEC for a block copolymer the individual block lengths cannot be determined but only the average chain length of the copolymer itself.

Another advantage of 2D-LC is to obtain an improvement in separation of the sample of interest which could not be obtained by the individual separation methods. This is possible because 2D-LC separation is directed by molecular weight in addition to chemical composition in the case where gradient HPLC is coupled with SEC (gradient-HPLC x SEC). This method has been successfully used by Kilz et al.[26] and Raust et al. [27]. Kilz et al. analysed

a four-arm star polymer based on poly(styrene-b-butadiene). The second step of the anionic polymerisation resulted in a mixture of linear, 2-arm, 3-arm and 4-arm species. The latter polymerisation was repeated three times, and each reaction mixture had different butadiene percentage. A mixture of all four reaction products, which resulted in a 16-component mixture, was then used for the 2D-LC analysis. The normal SEC analysis resulted in four poorly resolved peaks and the gradient HPLC analysis also resulted in poorly resolved peaks, but the combination of the two methods using SEC in the second dimension showed much improved results. The resolution increased significantly and a contour diagram clearly showing the complex mixture of the 16-component sample was obtained.

It is sometimes the case that identical chromatograms are obtained when using uncoupled methods, thus making it difficult to differentiate between samples. This problem is overcome when using 2D analysis, thus offering yet another advantage of 2D-LC. An example of such a situation is reported by Kilz[24].

Quantitative information can also be obtained with 2D-LC. For example molecular weight distribution MWD can be obtained when SEC is used in the second dimension and is calibrated in the usual way with suitable calibration standards. Functionality type distribution (FTD) can be calculated if the separation regarding functional groups in the first dimension is done, for example, with LCCC. It can then be calculated with the help of the PSS (Polymer Standard Service) 2D-LC software, where the volume of each peak from the contour plot is determined[24].

2.2.3. Detection and identification methods

There are different types of detectors and spectroscopy techniques that are often used for HPLC analysis and some of them also for 2D-LC analysis. The common detection and spectroscopy techniques are briefly discussed below.

The RI detector measures the difference in the refractive index of the effluent at the column outlet[28]. It will measure any differences in the refractive index of the sample to be analysed

and of the mobile phase. This is done by comparing the refractive index of the reference cell, containing a trapped sample of the mobile phase, to a second cell (sample cell) through which the mobile phase is flowing containing the analyte. This detector can detect any solute, has moderate sensitivity, is non-destructive, and the signal can directly be used as concentration signal. This detector cannot be used for gradient HPLC because it is very difficult to match the refractive indexes of the reference cell and the sample stream. The RI detector is also sensitive to temperature changes.

The ELSD is a very sensitive concentration detector. It detects non-volatile compounds and it can be used for isocratic or gradient analysis. The ELSD signal cannot be used directly as a concentration signal because it can be influenced by the sample chemistry and the chromatographic conditions but, after calibrating it, the signal can be used to obtain concentration information[29]. In the ELSD the mobile phase is evaporated and the light which is scattered by a non-volatile analyte is measured[28].

A UV detector is used as an on-line detector and it has a drawback which is that it is only helpful when the polymer samples contain UV active functional group(s), such as the aromatic groups in polystyrene. Another drawback of the UV detectors is that only solvents can be used that do not absorb UV radiation in the same region as the analyte. Usually solvents that do not have conjugated double bonds are most suitable[30]. The advantage of UV is the high sensitivity for aromatic compounds

FTIR has a similar drawback with regards to suitable solvent. The solvent used should not have the same functional groups that are used for the analysis of the analyte otherwise the functional groups of the solvent will obscure the ones of the analyte. Thus, in the case that a solvent is used for the chromatographic analysis that has the same functional group(s) as the analyte, then this solvent has to be evaporated first and then the sample has to be re-dissolved, but in a more suitable solvent. Therefore, FTIR is best used off-line instead of on-line. A way to overcome the solvent problem and also the time consuming evaporisation process LC-Transform is a very helpful interface system. Lab Connection Inc. introduced the LC-

Transform, which is a commercialized version of the heated nozzle technique which was discussed by Gagel and Biemann[31]. Before it was introduced, usage of SEC (HPLC)/FT-IR was limited due to the presence of mobile phase which first had to be removed before obtaining useful IR-spectra[32]. The heated nozzle technique was used to transfer the polymer fraction eluting from e.g. SEC or HPLC into a suitable form for FTIR analysis without affecting the elution profile or disturbing the integrity of the polymer [31,32]. From this method, data such as compositional distributions as a function of molecular weight can be obtained which gives important insight information for understanding the characteristics and performances of polymers. LC-transform is used as a direct SEC-FTIR[33] (or HPLC-FTIR) interface. More detailed information about LC-Transform can be found in references[32-34]. With FTIR, information about the chemical composition of the analyte can be obtained. It can also be used quantitatively, but for that a calibration is required which is relatively time consuming.

Proton and carbon NMR are also useful analytical methods but, compared to FTIR, they are very expensive and time consuming and the proton spectra can be quite complex and have a very low sensitivity.. The advantage is that no calibration is necessary for quantitative analyses.

On-flow and off-line detection have advantages and limitations. The advantages of on-flow detections are that the samples are not exposed to any contaminations or degradation due to solvent vaporisation. Its limitation is that the mobile phase, when not removed, might obscure the analytes' response signal. A disadvantage of the off-line detection is that sample handling and preparation is laborious and very time consuming, especially because it often involves solvent evaporation and some solvents are difficult to evaporate.

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Chapter 3

Experimental

3.1. Chemicals

3.1.1. Solvents for liquid chromatography

N,N-dimethylformamide (DMF, HPLC grade) obtained from Sigma-Aldrich (Sigma-Aldrich, Steinheim, Germany)

Tetrahydrofuran (THF, HPLC grade) obtained from Sigma-Aldrich (Sigma-Aldrich, Steinheim, Germany)

3.1.2. Polymer standards

Polystyrene (PS) calibration standards from different manufacturers (Polymer Standards Service (PSS) (Polymer Standards Service, Mainz, Germany), Polymer Laboratory (PL) (Polymer Laboratories, Church Stretton, Shropshire, UK) and Knauer (Berlin, Germany)) were used to have a well distributed range of different molecular weight PS standards, see **Table 3.1**.

Table 3.1: PS calibration standards used and the manufacturers.

M_p (g/mol)	Manufacturers
580	PL
700	Aldrich Chemical Company
1530	PL
2240	PSS-USA
6690	PSS-USA
10210	PL
17600	PSS-USA
29510	PL
39200	PSS-USA
72450	PL
92600	Knauer
117000	PSS-USA
170800	PL

538000	PL

Poly (ethylene oxide) (PEO) calibration standards of PL (lower molecular weight range) (Polymer Laboratories, Church Stretton, Shropshire, UK) and PSS (higher molecular weight range) (Polymer Standards Service, Mainz, Germany) were used, see **Table 3.2** below.

Table 3.2: PEO calibration standards used and the manufacturers.

M_p (g/mol)	Manufacturer
440	PL
600	PL
1470	PL
4100	PL
7100	PL
12600	PL
23600	PL
44700	PSS
62000	PSS
114000	PSS
178000	PSS
289000	PSS

3.1.3. Copolymers

The PS-b-PEO copolymers are from PSS (Polymer Standards Service, Mainz, Germany) and the sample details from the supplier are as follows:

Table 3.3: Block copolymer sample details according to the manufacturer.

PS-b-PEO	M_w total (g/mol)	M_w PS (g/mol)	M_w PEO (g/mol)
1	-	M_n = 1500	M_n = 3170
2	-	M_n = 1500	M_n = 3960
3	7090	3940	3150
4	31930	2930	29000

5	60000	30000	30000
6	91500	30000	61500
7	134000	30000	104000
8	218000	109000	109000

3.2. Chromatographic system

3.2.1. Liquid chromatography at critical conditions

For all the experimental work done to establish critical conditions of PS and PEO and for all the analysis at these conditions a HPLC system was used consisting of the following units: Waters 2690 Separation module (Waters, Milford, MA, USA); Agilent 1100 Series variable wavelength UV-Vis detector (Agilent Technologies, Waldbronn, Germany); PL-ELS 1000 detector (Polymer Laboratories, Church Stretton, Shropshire, UK); data recording and processing using PSS WinGPC Unity (Build 5403) software (Polymer Standards Service, Mainz, Germany).

The conditions used for the critical conditions of PS are as follows: a mobile phase composition of THF:DMF 18:82 vol.% with a flow rate of 0.5 mL/min. A C18 modified silica column was used (Symmetry 300 Å, 5 µm, 4.6 x 250 mm, Waters, Milford, MA, USA) at 30ºC. PS calibration standards were used to establish the critical conditions. The solvents were pre-mixed and used as mobile phases as well as solvents for samples and standards at a concentration of approximately 5 mg per 1.5 mL. To dissolve the samples they were heated to 40-45ºC.

The conditions used for the critical conditions of PEO are as follows: a solvent composition of DMF:THF 4:96 vol.% with a flow rate of 0.5 mL/min. A silica column was used (Nucleosil 300 Å, 5 µm, 4.6 x 250 mm, Macherey-Nagel, Düren, Germany) at 29.7ºC. PEO calibration standards were used to establish the critical conditions. The solvents were pre-mixed and used as mobile phases as well as solvents for samples and standards at a concentration of approximately 5 mg per 1.5 mL. To dissolve the samples they were heated to 40-45ºC.

The copolymer sample solutions were prepared the same way as the PS and PEO standards were prepared.

ELSD settings for LCCC runs were used as suggested by the supplier: 180ºC for evaporation and 80ºC for nebulisation at a N_2 gas flow rate of 1.5 SLM (standard litres per minute).

Some of the copolymers were manually fractionated at critical conditions of PS and PEO, using a concentration of approximately 15 mg per 1.5 mL.

3.2.2. Calibration of the ELSD with PS and PEO calibration standards

Different molecular weight PS calibration standards were dissolved as described above at a concentration of 5 mg per 1.5 mL. Different injection volumes of each PS solution were injected and run at the critical conditions of PS using the same ELSD settings as mentioned above.

Similar procedure as with PS calibration standards was followed to establish a calibration curve for the ELSD using different molecular weight PEO calibration standards and using critical conditions of PEO.

Both these calibration processes were done in one dimensional setup.

3.2.3. Size exclusion chromatography

For SEC as the second dimension in 2D-LC a PSS GRAM HighSpeed 1000 Å column (Polymer Standards Service, Mainz, Germany) with DMF as mobile phase was used. The column was kept at room temperature and the flow rate used was 2.5 mL/min. Comparing THF and DMF the latter was the only solvent which dissolved PS and PEO calibration standards as well as the block copolymers therefore it was used the mobile phase for the second dimension.

The ELSD settings where established by injecting a PS standard with a given concentration at different evaporation and nebulisation temperatures and comparing the peak areas. When the

area decreases this indicates that the temperature settings are too high. Following that procedure the appropriate ELSD settings were found to be 230ºC for evaporation and 130ºC for nebulisation and a N_2 gas flow rate of 1.5 SLM at a DMF flow rate of 2.5 mL/min. The pump used was a Waters 515 HPLC pump.

3.2.4. Two-dimensional liquid chromatography

For the combination of LCCC (for PS and PEO) and SEC (conditions used as described in **Section 3.2.1 and 3.2.3** respectively) an eight-port electrically driven switching valve with two 100 µL sampling loops was used. The flow rate for the first dimension was set to be 0.02 mL/min and for the second dimension 2.5 mL/min and the ELSD settings were as described in **Section 3.2.3**.

The copolymer concentration was 20 mg per 1.5 mL of mobile phase of the respective critical conditions.

The second dimension was calibrated, once with PS and once with PEO in DMF. A wide range of different PS calibration standards were dissolved in DMF and heated to 40-45ºC. Each of the dissolved PS calibration standards were than directly injected into the second dimension. The settings for the second dimension are the same as mentioned above. The same procedure was followed to establish the calibration curve for PEO. For the molecular weight determination the PS and PEO calibration curves were applied to their corresponding critical conditions. The two calibration curves are shown in **Figure 3.1**.

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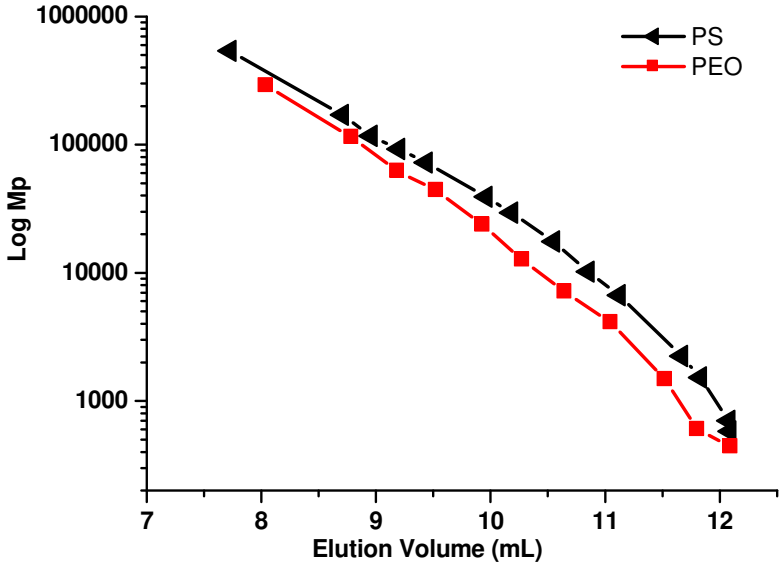

Figure 3.1: PS and PEO calibration curves for SEC in the second dimension of 2D-LC.

For the 2D plots the same software was used which was used for all the HPLC analysis for this study

3.3. FTIR

3.3.1. Reflectance FTIR for qualitative analysis

The reflectance FTIR analysis was carried out on a Thermo Scientific Nicolet iN10 FTIR microscope (Waltham, MA, USA) using the Omnic 8.1.10 software.

The original block copolymers and all the fractions of selected samples were dissolved in DMF and heated at least for approximately an hour at about 40ºC before they were prepared for FTIR analysis. The gold plates were prepared by spotting the dissolved samples onto them and then they were left for several hours in the fume cupboard for DMF to evaporate followed by a final drying session in the vacuum oven over night at room temperature before any spectra were collected. Several spectra for each sample spot were collected of which the best spectra were then picked.

3.3.2. Solution cell FTIR for quantitative analysis

Quantitative FTIR analysis was carried out on a Thermo Scientific Nicolet iS10 FTIR spectrometer (Waltham, MA, USA) using a solution cell with Zinc selenide (ZnSe) windows (32 x 3 mm) (Pike Technologies (Spectroscopic Creativity),Madison, WI, USA). Path length was fixed to 0.025 mm using a Teflon spacer.

To establish a PS/PEO FTIR calibration curve, stock solutions of PS 10210 g/mol and PEO 12600 g/mol with a concentration of 5 mg/mL were made up. Both solutions were heated for approximately one hour at 40ºC. From these stock solutions mixtures with different ratios of PS and PEO were made up of which spectra were obtained. Of these obtained spectra the reciprocal area ratios of the peaks at the frequency of 700 cm^{-1} for PS and 1140 cm^{-1} for PEO were used to establish the calibration curves as can be seen in **Figure 3.2**.

The block copolymers and all the first fractions of selected samples were dissolved in DMF and heated at least for an hour at approximately 40ºC before FTIR analysis. Before the spectra were collected, for each sample a DMF background was collected which was than subtracted from samples spectra. The reciprocal peak area ratio from the above mentioned peak frequencies were determined if present. With the help of the calibration curve in **Figure 3.2** the percent content of PS and PEO of the original samples as well as of the first fractions of selected samples was determined.

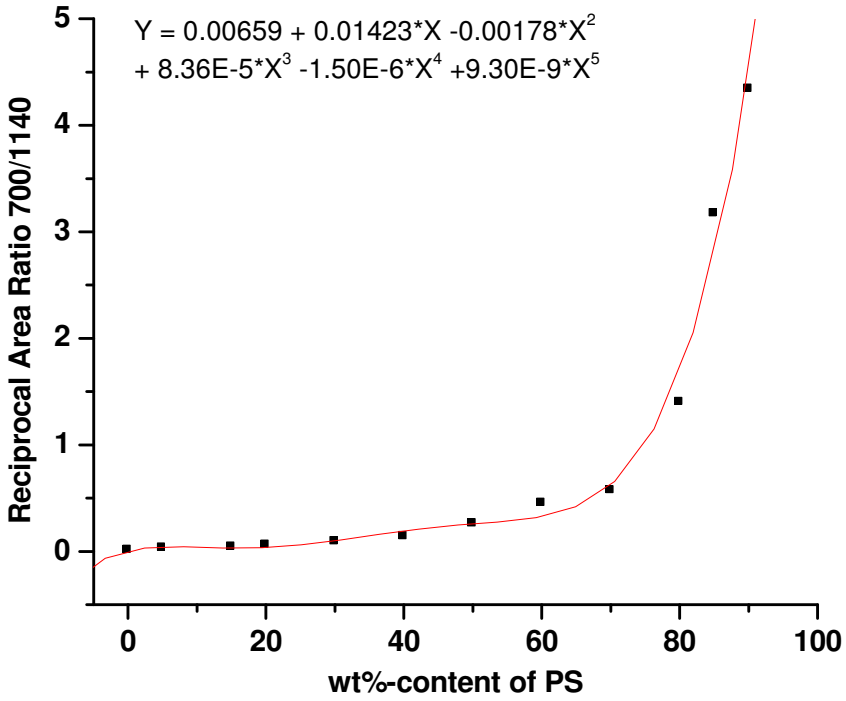

Figure 3.2: FTIR calibration curve using reciprocal area ratio of the peak at the frequency of 700 cm^{-1} for PS and 1140 cm^{-1} for PEO.

Chapter 4

Results and discussion

4.1. Introduction

The analysis of a polystyrene-block-poly(ethylene oxide) (PS-b-PEO) copolymer is, as mentioned in **Chapter 2**, more complex than the analysis of a random styrene-ethylene oxide copolymer since it has a sequence length distribution in addition to the global molar mass and chemical composition distributions. The objectives of this project were to determine the chemical composition distribution of the copolymers, the block length of each block in the copolymers, as well as the respective homopolymer content. The best way to approach this analysis was to establish critical conditions for each block type of the copolymer. Thus one of the blocks was made "chromatographically invisible" and the length of the other block was determined and vice versa. At the critical conditions of a specific part of a copolymer ("chromatographically invisible" part) all the molecules (of the same chemical composition) elute at the same elution volumes independent of molecular weight. While operating at the critical conditions of one part of the copolymer (e.g. PS) the other part will either elute in the SEC or the LAC mode depending on factors such as the polarity of the stationary phase, polarity of the polymer, the operating temperature and the solvent composition used. To determine the molecular weights of possible homopolymers as by-products, two-dimensional chromatography was used, where liquid chromatography at critical condition (LCCC) in the first dimension was coupled to size exclusion chromatography (SEC) in the second dimension. The chemical composition of the fractions was determined by Fourier-transform infrared spectroscopy (FTIR) spectroscopy.

4.2. FTIR analysis

The FTIR spectra of polystyrene (PS) and poly(ethylene oxide) (PEO) calibration standards are presented in (**Figure 4.1**). When comparing the two spectra the main peaks to be used for the determination of the presence of either component (PS or PEO) are at wave numbers of approximately 700 cm^{-1} (aromatic C-H bend) and 3025 cm^{-1} (aromatic C-H stretch) for PS (circles) and at 2880-3000 cm^{-1} (C-H stretch), 3300-3600 cm^{-1} (O-H stretch) as well as the range of 840 – 1360 cm^{-1} (C-O-C stretch) for PEO (rectangles) where the bands encircled with the solid circle were used for quantification purposes.

When examining the spectra of the eight copolymers (**Figure 4.2** to **Figure 4.5**) it can be seen that all samples exhibit the characteristic absorption peaks for PS and PEO.

Table 4.1 presents the calculated chemical compositions for all eight copolymer samples. These results were determined with the help of a calibration curve. In **Section 3.3.2** it was described how this calibration curve (**Figure 4.2**) was established. It needs to be said that it was quite a difficult task to find an appropriate peak for PEO which could be used for the calibration curve because N,N-dimethylformamide (DMF) was used as a solvent. As can be seen in **Figure 4.1** DMF exhibits strong absorption bands in the region of the PEO absorptions. When DMF is subtracted as background from the samples spectra than hardly any useful strong PEO peaks are left to be used for the calibration curve. At first it was decided to use the peak area at wave number of 950 cm^{-1} but no steady decrease in peak area was observed as the PEO content of the PS-PEO blend decreased, but they rather were random. Therefore it was decided to rather use the peak area at a wave number of 1140 cm^{-1}. The peak showed at least the expected trend of decreasing peak area as the PEO content of the PS-PEO blend decreased. As it can be observed in the spectrum of PEO (**Figure 4.1**) that peak at 1140 cm^{-1} is not very strong and therefore the calibration curve (**Figure 3.2)** might not be very reliable but at least a rough indication of the chemical compositions for the eight copolymers could be obtained. When comparing now the obtained results with those of the manufacturers it can be seen that both results are in fair agreement.

Even though it is confirmed that there is PS and PEO present in each copolymer sample, FTIR does not give any information if the samples are copolymers or just blends of the two homopolymers. Therefore further analysis is necessary.

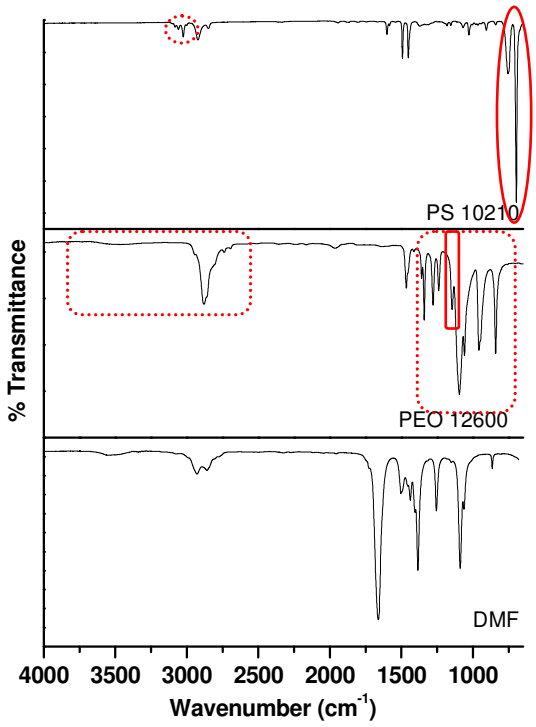

Figure 4.1: FTIR spectra of PS 10210 g/mol, PEO 12600 g/mol calibration standards and DMF solvent.

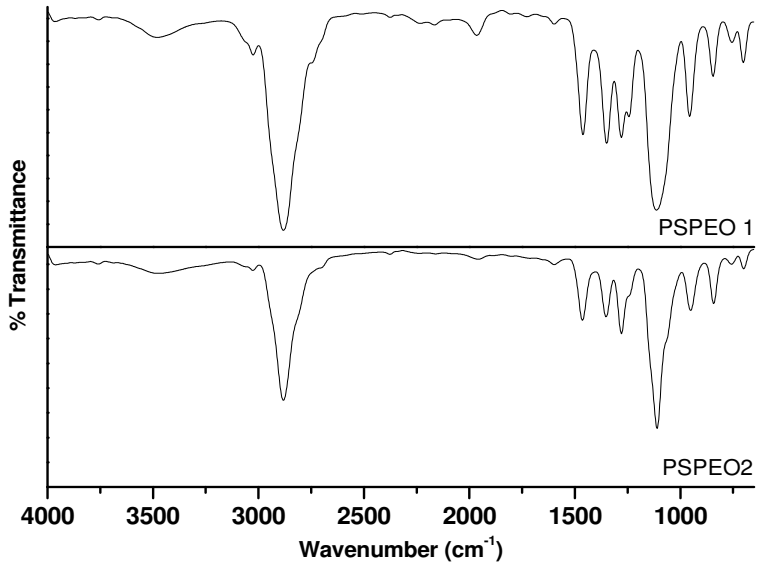

Figure 4.2: FTIR spectra of PS-b-PEO 1 and PS-b-PEO 2.

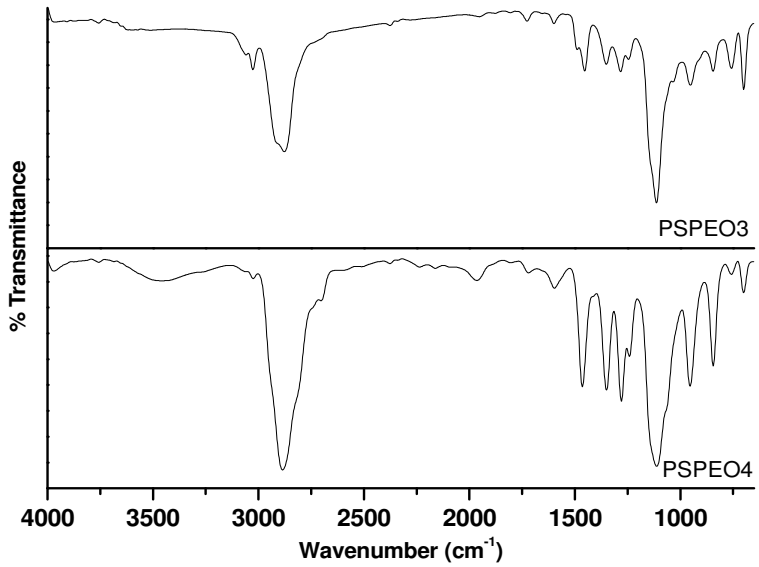

Figure 4.3: FTIR spectra of PS-b-PEO 3 and PS-b-PEO 4.

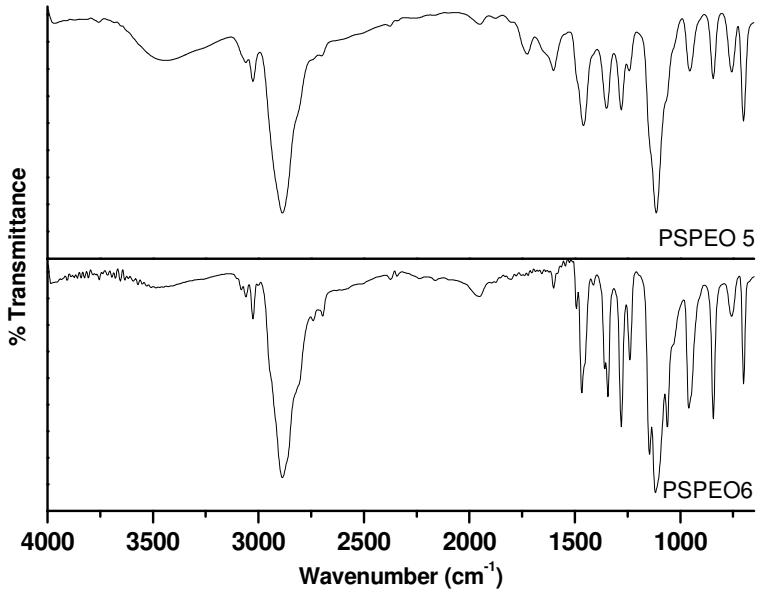

Figure 4.4: FTIR spectra of PS-b-PEO 5 and PS-b-PEO 6.

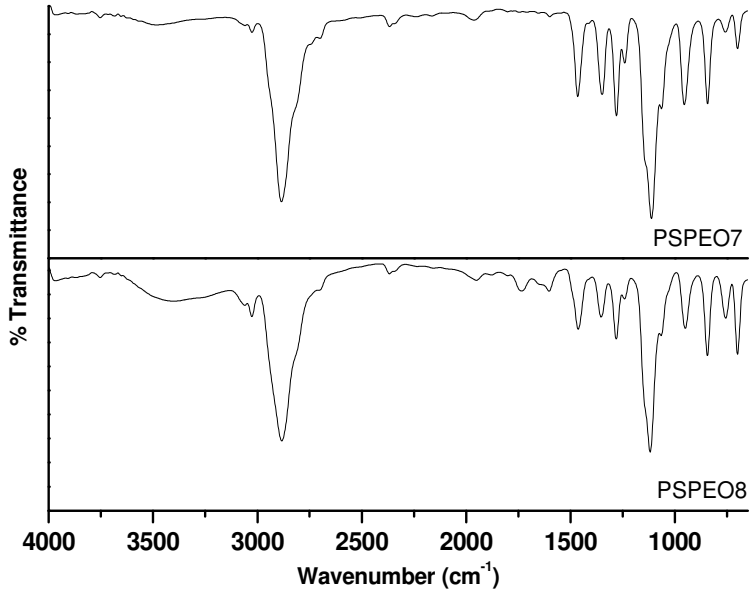

Figure 4.5: FTIR spectra of PS-b-PEO 7 and PS-b-PEO 8.

Table 4.1: Chemical composition of the block copolymer samples determined with solution cell FTIR compared to manufacturer's data.

	FTIR results		Manufacturer results	
	PS content (wt.%)	PEO content (wt.%)	PS content (wt.%)	PEO content (wt.%)
PS-b-PEO 1	33	67	32	68
PS-b-PEO 2	31	69	27	73
PS-b-PEO 3	66	34	56	44
PS-b-PEO 4	2	98	9	91
PS-b-PEO 5	65	35	50	50
PS-b-PEO 6	31	69	33	67
PS-b-PEO 7	28	72	22	78
PS-b-PEO 8	50	50	50	50

4.3. Critical conditions of PS

For the critical conditions of PS, THF-water was used first and it was noted that that solvent system was not optimal as discussed below. Therefore another solvent combination was tried, namely THF-DMF which show better results as discussed in **Section 4.3.2**.

4.3.1. Critical conditions of PS with THF-water

THF (good solvent, which promotes desorption of the sample from C18 modified stationary phase) and water (poor solvent, which promotes adsorption of the sample to the C18 modified stationary phase) was used. Baran et al.[4] used THF-water as the mobile phase to establish the critical conditions of PS in order to analyse relatively low molecular weight PS-b-PEO. They found the critical conditions to be THF:H_2O (87.1:12.9 wt.%) using a Nucleosil C18 100 Å column. For the present study a Symmetry C18 300 Å column was used and different ratios of THF:H_2O from (87:13 vol.%) to (90:10 vol.%) were pre-mixed. Pre-selected PS calibration standards were dissolved in the different pre-mixed solvent compositions. The results for the different solvent compositions are shown in **Figure 4.6**. As can be seen the PS calibration standards elute in the SEC mode (lower elution volume (V_e)) when using a THF:H_2O composition of 90:10 vol.%. When using THF: H_2O 87:13 vol.% the PS calibration standards elute in the liquid adsorption chromatography (LAC) mode (higher V_e).The solvent composition of THF:H_2O 88.5:11.5 vol.% corresponds to the critical conditions. As can be observed, up to a molecular weight at the peak maximum (M_p) of 33000 g/mol the calibration standards elute at nearly the same V_e, in other words at the critical conditions. A M_p of 33000 g/mol was the highest molecular weight to be used because the stationary phase had a small average pore size of 100 Å and an exclusion limit of about 30000 g/mol. However, solubility in this solvent composition was achieved for much higher molecular weights.

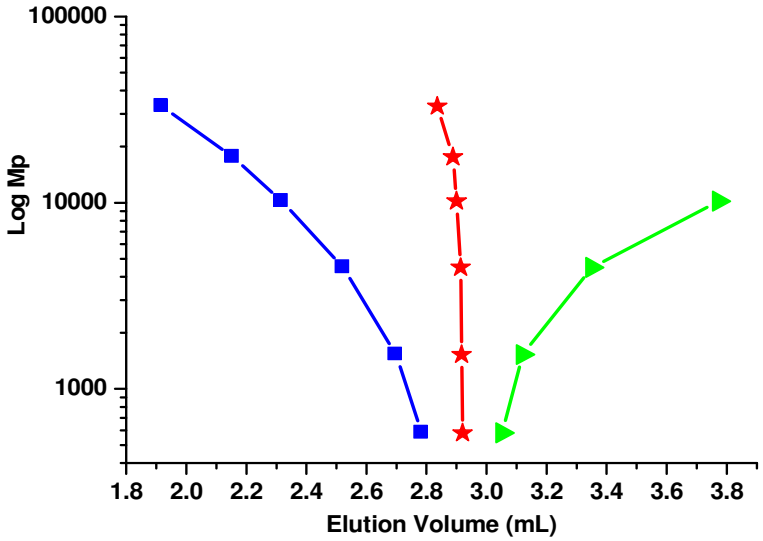

Figure 4.6: Plots of log M_p vs. V_e of PS at different THF:H_2O ratios, ▶= 87:13, ★ = 88.5:11.5, and ■ = 90:10 vol.%. Column: 100Å C18 Summetry, 4.6 x 250 mm at 30°C.

Under the same experimental conditions PEO calibration standards of different molecular weights were investigated, see **Figure 4.7**. A M_p of approximately 23600 g/mol of PEO was the maximum that could be dissolved in the present mobile phase composition. **Figure 4.7** shows that the PEO calibration standards elute in the order of decreasing molecular weight. This indicates that at critical conditions of PS, PEO elutes in the SEC mode. It was rather unexpected to see that the lowest molecular weight PEO standard eluted significantly earlier than the lowest molecular weight PS standard. This could be due to the fact that the PS standards have non-polar alkyl end groups which interact with the non-polar stationary phase.

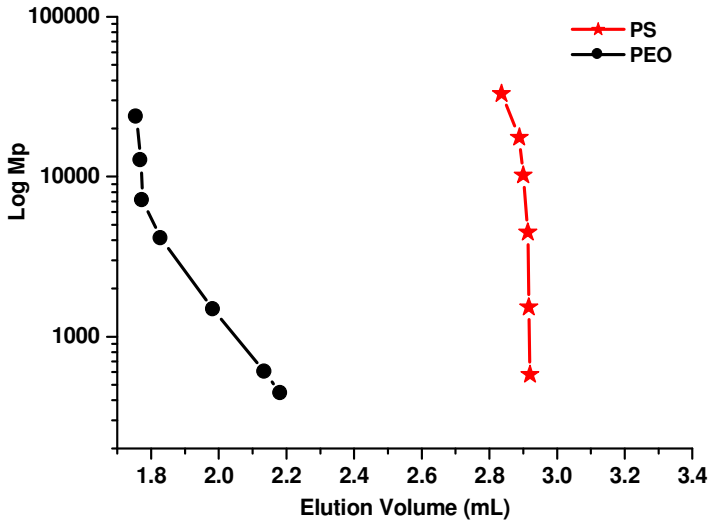

Figure 4.7: Plots of log M_p vs. V_e of PS and PEO at critical conditions of PS with THF:H_2O at a ratio of 88.5:11.5 vol.%. Column used: 100 Å C18 Summetry, 4.6 x 250 mm at 30°C.

In order to evaluate the separation capability of the present stationary phase at the critical conditions of PS, different blends of PS and PEO with similar M_p were dissolved and run at the established conditions. As can be seen in **Figure 4.8** (B and D), the two different PEO-PS blends were baseline separated irrespective of the fact that their molecular weights are quite close to each other. The black box shows the PS with different M_p eluting at the same V_e, denoting that elution takes place independent of molecular weight. The PEO with different M_p elute in the order of decreasing M_p (proving SEC mode), as the dotted arrow indicates. In **Figure 4.8** there are both the evaporative light scattering detector (ELSD) (B and D) and the ultraviolet (UV) signals (A and C) shown for the corresponding blends. The UV detector, operating at a wavelength of 254 nm, detects only PS (UV active phenyl group) and not PEO (no UV active elements), whereas the ELSD detects both PS and PEO. Therefore the UV signal is a confirmation for the presence of PS.

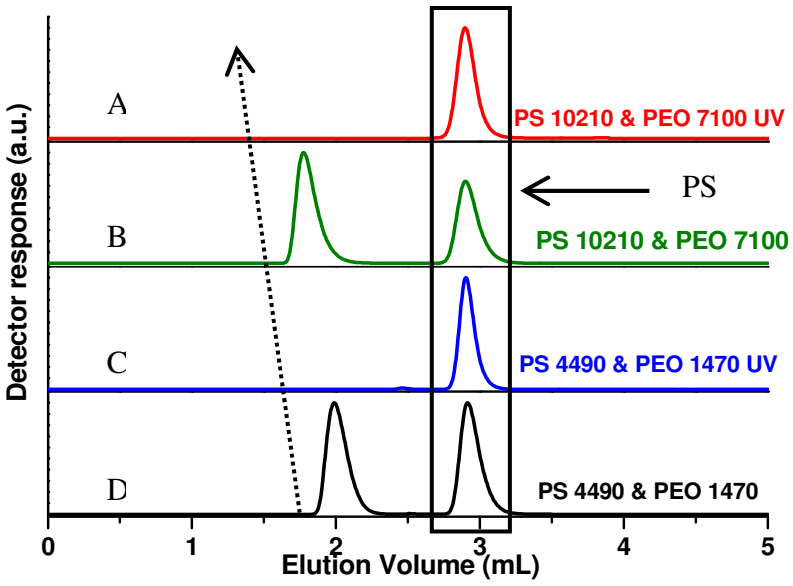

Figure 4.8: Blends of PS and PEO calibration standards run at the critical conditions of PS (THF:H_2O 88.5:11.5 vol.%). Shown are the ELSD (B,D) and the corresponding UV-254 nm signals (A,C).

The chromatogram of PS-b-PEO 1 plotted in **Figure 4.9** (A) shows a small peak (encircled) which is tentatively assigned to PS homopolymer. To confirm the presence of this homopolymer a small amount of PS calibration standard 10210 g/mol was added. This process is termed spiking the sample. The resulting chromatogram is plotted in **Figure 4.9** (B). As can be seen in the spiked sample, the PS calibration standard elutes at a similar V_e as the PS homopolymer in PS-b-PEO 1. Therefore it proves that there is PS homopolymer present in the sample PS-b-PEO 1.

The separation of PS-b-PEO 3, PS-b-PEO 3 spiked with PS (10210 g/mol), and the corresponding PS calibration standard are plotted in **Figure 4.10** (from top to bottom respectively, use A, B, C).The chromatograms indicate that this sample does not contain PS. On the other hand, there is a shoulder on the lower V_e side of the main peak which could be due to the presence of PEO homopolymer. This will be analysed at a later stage.

Figure 4.11 shows the sample PS-b-PEO 5 (A) as well as the PS calibration standard (B) with a molecular weight of 39200 g/mol. For the copolymer the corresponding UV-254 nm signal

was added to see the PS content. The ELSD signal of PS-b-PEO 5 (A) shows a non-baseline separated trimodal peak. The peak with the larger V_e might correspond to PS homopolymer, which elutes close to the V_e of the pure PS calibration standard (B which is the first confirmation that this peak is PS homopolymer. The second confirmation is the UV trace, since the UV detector can only detect PS as mentioned above. With the help of the UV traces it can be said that the middle peak is diblock copolymer because it shows the presence of PS. The peak at the lowest V_e does not show any UV activity and, therefore, must be due to PEO homopolymer. Apparently the PEO homopolymer has a slightly higher molecular weight than the corresponding PEO block in the block copolymer because it elutes at a lower V_e than the copolymer.

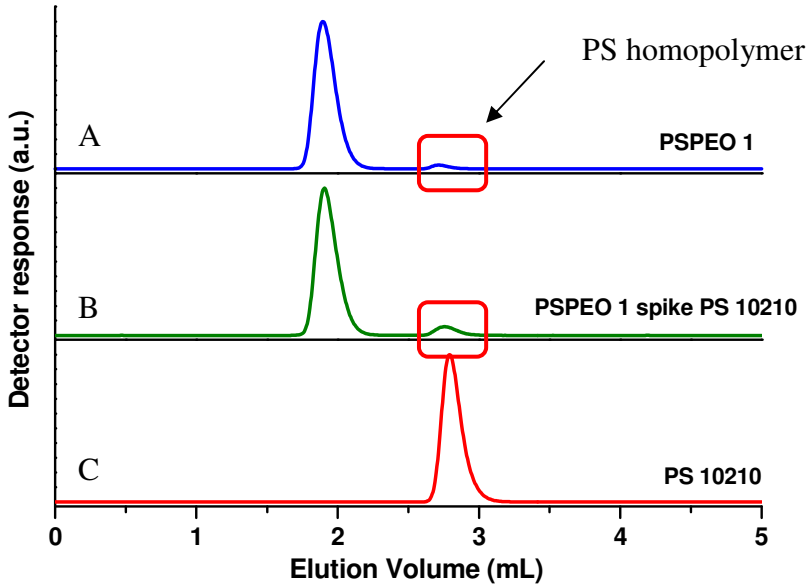

Figure 4.9: PS-b-PEO 1 (M_w of PS 1500 g/mol and M_w of PEO 3170 g/mol), PS-b-PEO 1 spiked with PS 10210 and PS 10210 run at the critical conditions of PS (THF:H_2O 88.5:11.5 vol.%).

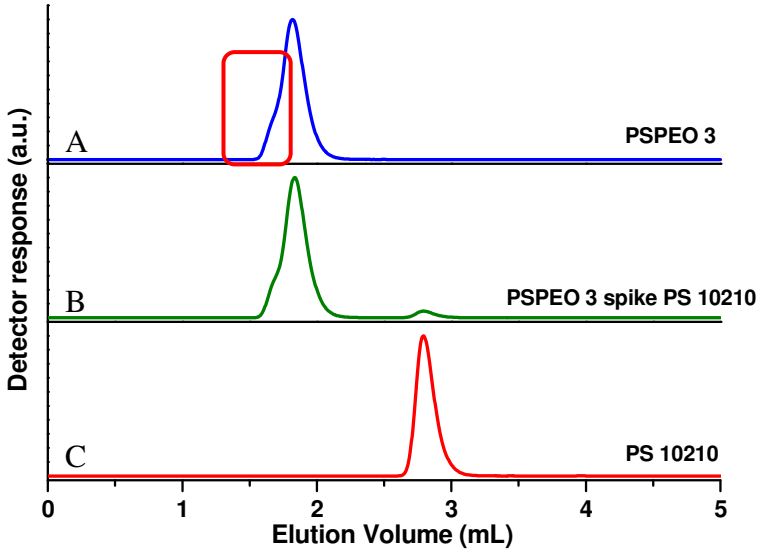

Figure 4.10: PS-b-PEO 3 (M_w of PS 3940 g/mol and M_w of PEO 3150 g/mol), PS-b-PEO 3 spiked with PS 10210 and PS 10210 run at the critical conditions of PS (THF:H_2O 88.5:11.5 vol.%).

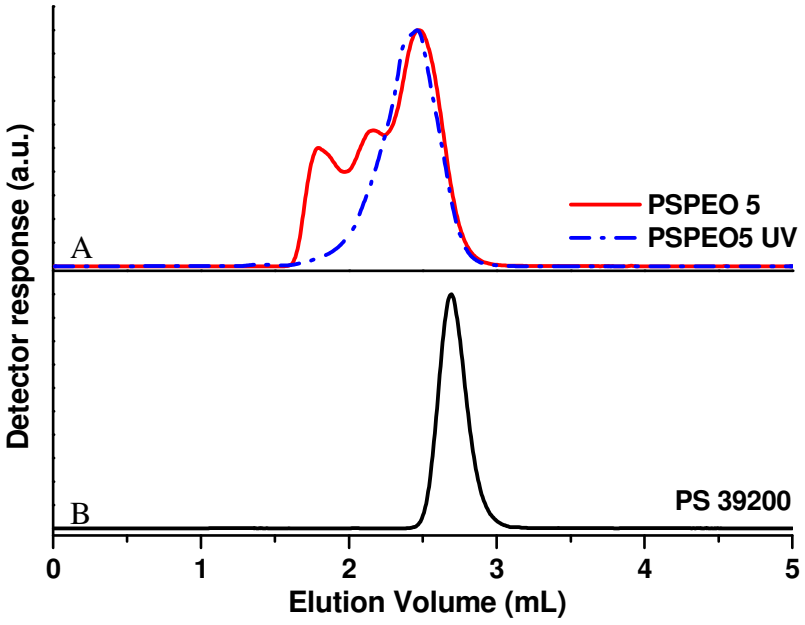

Figure 4.11: PS-b-PEO 5 (M_w of PS 30000 g/mol and M_w of PEO 30000 g/mol) with the ELSD (A,B) and UV-254 nm signal (A -.-) and PS calibration standard 39200 g/mol run at critical conditions of PS (THF:H_2O 88.5:11.5 vol.%).

Table 4.2 shows the V_e of the peak maxima for PS-b-PEO 1, 3 and 5 and what their molecular weight would be according to the PEO calibration curve in **Figure 4.7**. These results are then compared to the molecular weight data from the manufacturer. As can be seen for those three samples the molecular weight for the PEO block in the block copolymer is not in agreement with those indicated by the manufacturer. In average, the molecular weight for the PEO block is less than the manufacturer indicated.

Table 4.2: Comparison of the obtained V_e and their corresponding M_p (according to PEO calibration curve from Figure 4.7) with the manufacturer's M_w.

	M_p of copolymer	**PEO calib.**	**Manuf. data**
	V_e (mL)	M_p (g/mol)	M_w (g/mol)
PS-b-PEO 1	1.89	2020	3170
PS-b-PEO 3	1.82	4510	3150
PS-b-PEO 5	1.79/2.17	7820/480	30000

4.3.2. Critical conditions of PS with THF-DMF

Due to the poor solubility in THF/water of PEO above 23600 g/mol it was decided to investigate another solvent system which would work better. Berek[5] used a THF-DMF solvent system with different stationary phases. He was able to dissolve PEO calibration standards way above the molecular weight limit that was achieved in this project with the THF-H_2O solvent composition.

The critical conditions of PS was established using a C18 modified stationary phase with THF (good solvent) and DMF (poor solvent) as mobile phase composition. Different ratios of THF:DMF from (20:80 vol.%) to (17:83 vol.%) were pre-mixed. Pre-selected PS calibration standards where dissolved in the different pre-mixed solvent compositions.

The results of using the different solvent compositions are shown in **Figure 4.12**. As can be seen the PS calibration standards elute in the SEC mode (lower V_e) when using a THF:DMF composition of 20:80 vol.%. When using 100 vol.% DMF the PS calibration standards elute in LAC mode (higher V_e).The solvent composition of 18:82 vol.% (THF:DMF) was the closest

to the critical conditions that could be obtained. As can be observed, up to a M_p of 39200 g/mol the calibration standards elute at nearly the same V_e. For the higher molecular weights V_e decreases slightly indicating that these standards do not elute exactly at critical conditions. For this study, however, a further adjustment of the critical conditions was not conducted.

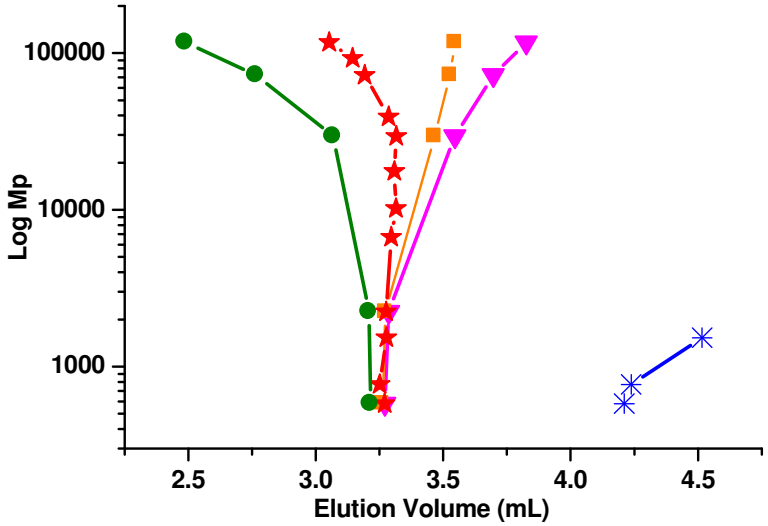

Figure 4.12: Plot of log M_p vs. V_e of PS at different THF:DMF ratios, ✳ = 0:100, ▼ = 17:83, ■ = 17.5:82.5, ★ = 18:82, and ● = 20:80 vol.%. Column: 300 Å C18 Symmetry, 4.6 x 250 mm at 30°C.

In **Figure 4.13** the PEO calibration curve is presented that was obtained at chromatographic conditions corresponding to the critical conditions of PS. As can be seen, proper separation is achieved up to a molecular weight of about 50000 g/mol for PEO, above that molecular weight the exclusion limits is reached. In other words the polymer molecules are excluded from the pores of the stationary phase. This calibration should allow determining the block lengths of the PEO blocks in the block copolymers. At higher molecular weights the stationary phase reaches its exclusion limit.

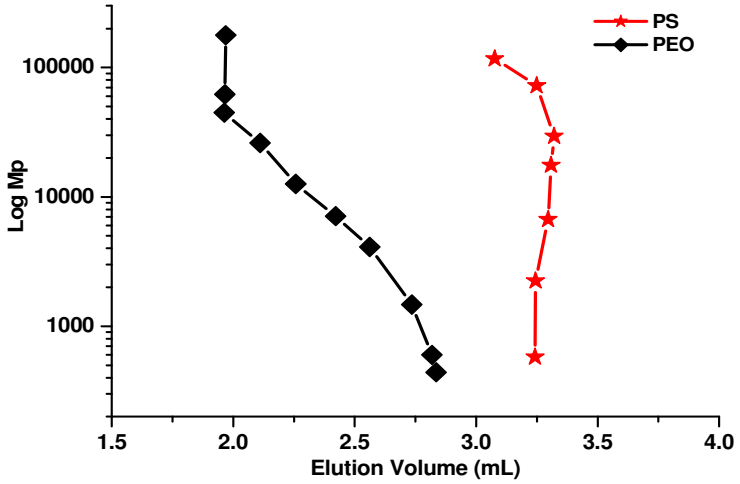

Figure 4.13: Plot of log M_p vs. V_e of PS and PEO at critical conditions of PS with THF:DMF at a ratio of 18:82 vol.%. Column: 300 Å C18 Symmetry, 4.6 x 250 mm at 30°C.

4.3.3. LCCC method development

To test the separation at the critical conditions of PS with the current mobile phase composition, different blends of PS and PEO with similar molecular weights were investigated. As can be seen in **Figure 4.14**, components of the three different blends were baseline separated. The black box shows PS with different molecular weights eluting at the same V_e, indicating that they elute irrespective of their molecular weight at critical conditions. The PEO calibration standards with different molecular weights elute in the order of decreasing molecular weights as the dotted arrow is showing.

The chromatograms of PS-b-PEO 1 and PS-b-PEO 2 plotted in **Figure 4.15** show the presence of some PS homopolymer (small encircled shoulders). There is not much difference between the two block copolymers and from the manufacturer's data it is known that PS-b-PEO 2 only has a little higher PEO content. The main peak corresponds to the copolymer, which might also contain some PEO homopolymer. For simplicity these peaks will be referred to as block copolymer for now, however, the situation will be studied more in detail in the forthcoming sections.

When comparing the chromatograms of PS-b-PEO 1 analysed with the THF-H_2O system with those of the THF-DMF system, it can be observed that the THF-H_2O system gave a much better separation between the copolymer and the PS homopolymer.

To prove the presence of PS homopolymer, PS-b-PEO 1 was spiked with PS (2240 g/mol). In **Figure 4.16** the original sample (A), the spiked sample (B) and the PS 2240 g/mol (C) are plotted. As can be seen in the spiked sample (B), the PS calibration standard elutes close to the position of the previously detected shoulder. This proves the assumption that there is PS homopolymer present in PS-b-PEO 1. The reason that the V_e of PS peak in the spiked sample (B) is not exactly the same as the V_e of the shoulder in the original sample (A) is that the homopolymer present in the copolymer sample most probably has different end groups than the PS calibration standards which were used to establish the critical conditions.

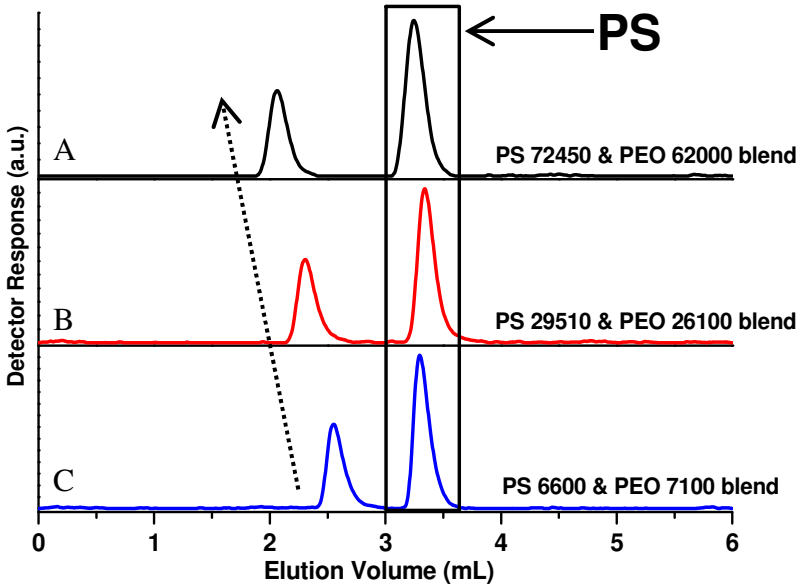

Figure 4.14: Blends of PS and PEO calibration standards run at the critical conditions of PS (THF:DMF 18:82 vol.%).

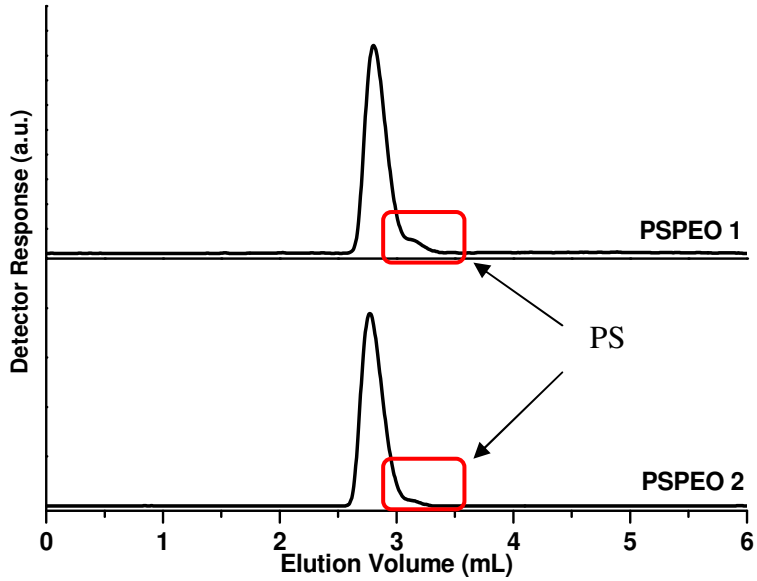

Figure 4.15: PS-b-PEO 1 (M_w of PS 1500 g/mol and M_w of PEO 3170 g/mol) and PS-b-PEO 2 (M_w of PS 1500 g/mol and M_w of PEO 3960 g/mol) run at critical conditions of PS (THF:DMF 18:82 vol.%).

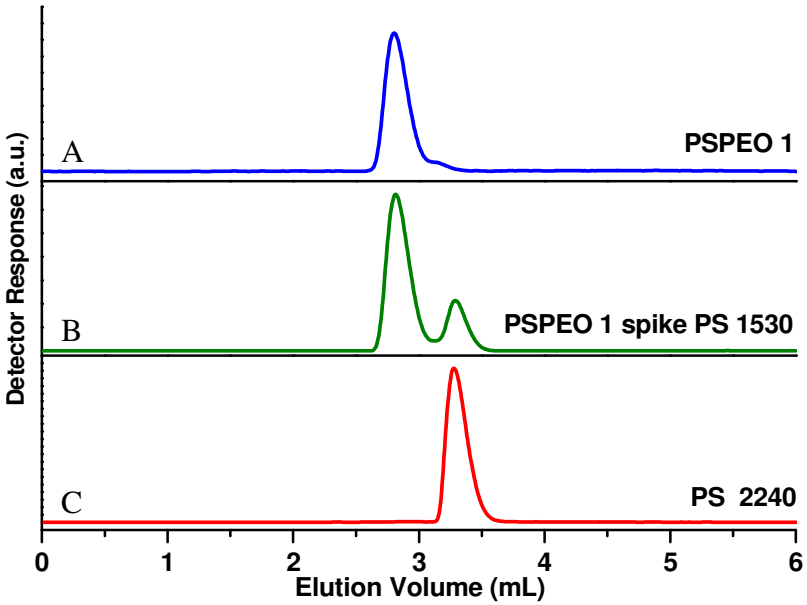

Figure 4.16: PS-b-PEO 1, PS-b-PEO 1 spiked with PS 2240 and PS 2240 run at the critical conditions of PS (THF:DMF 18:82 vol.%).

PS-b-PEO 3, 4 (**Figure 4.17**), 6 and 7 (**Figure 4.18**) are relatively similar. They all show the presence of copolymer while PS homopolymer is not detected. PS-b-PEO 3 and PS-b-PEO 7 have a clearly visible shoulder (encircled). These shoulders could be due to the presence of

PEO homopolymer which might have a slightly different molecular weight compared to the PEO block in the block copolymer and therefore elutes so closely. The origin of the shoulder will be investigated at a later stage. When comparing the V_e at peak maximum of the four samples an overall decrease can be noticed that is due to an increase in molecular weight of the PEO blocks. PS-b-PEO 4 and 6 elute at approximately the same V_e indicating that they have very similar PEO block lengths.

PS-b-PEO 7 was spiked with a PS calibration standard (29510 g/mol). As it can be deduced from chromatogram B in **Figure 4.18** the V_e of the PS in the spiked sample has no corresponding peak in the pure PS-b-PEO 7 (A) thus proving that there is no PS homopolymer present in that sample.

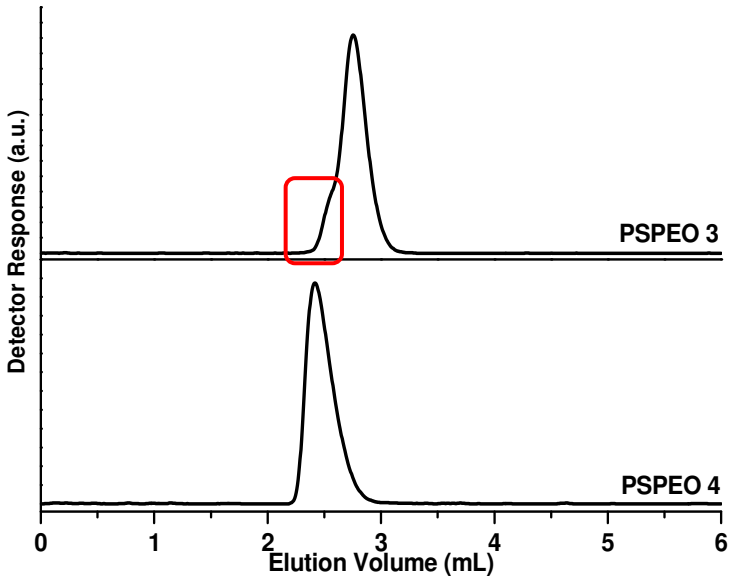

Figure 4.17: PS-b-PEO 3 (M_w of PS 3940 g/mol and M_w of PEO 3150 g/mol) and PS-b-PEO 4 (M_w of PS 2930 g/mol and M_w of PEO 29000 g/mol) run at critical conditions of PS (THF:DMF 18:82 vol.%).

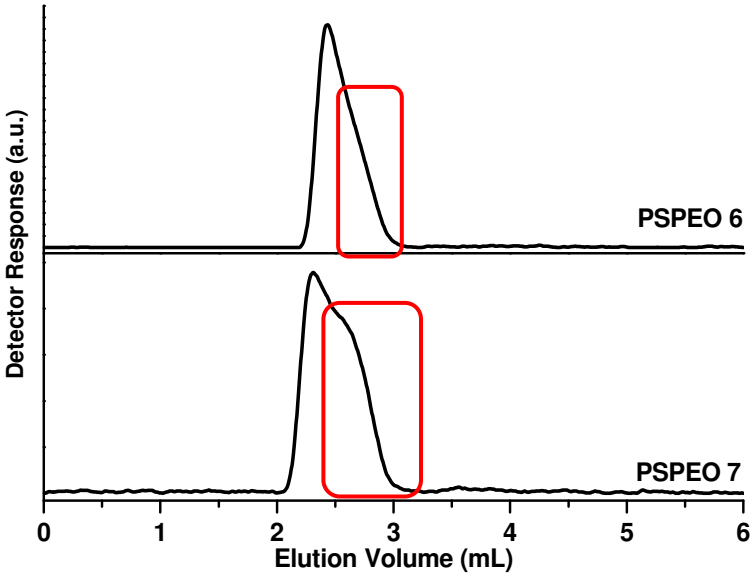

Figure 4.18: PS-b-PEO 6 (M_w of PS 30000 g/mol and M_w of PEO 61500 g/mol) and PS-b-PEO 7 (M_w of PS 30000 g/mol and M_w of PEO 104000 g/mol) run at critical conditions of PS (THF:DMF 18:82 vol.%).

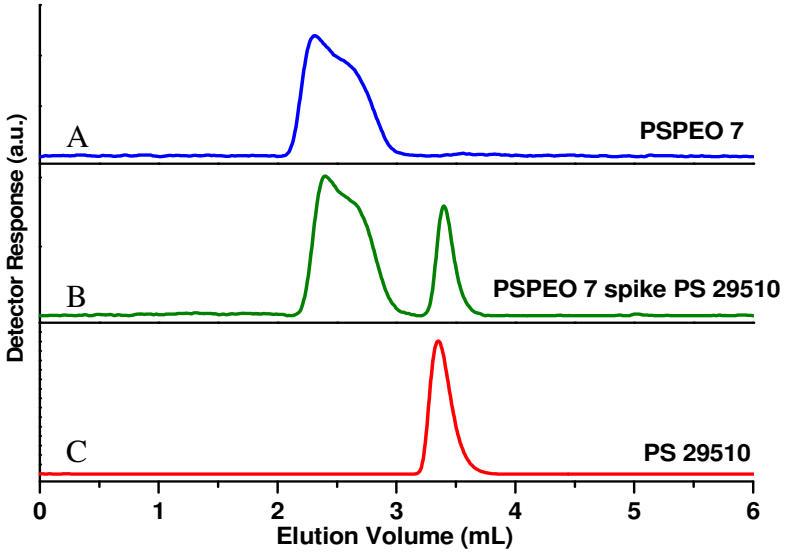

Figure 4.19: PS-b-PEO 7, PS-b-PEO 7 spiked with PS 29510 and PS 29510 run at the critical conditions of PS (THF:DMF 18:82 vol.%).

PS-b-PEO 5 and 8 (**Figure 4.20**) both contain significant amounts of PS homopolymer in addition to the copolymer. They are the most heterogeneous of all samples. The copolymer fractions elute in very broad peaks indicating that their chemical heterogeneity is large.

Comparing the V_e of the copolymer fractions in the peak maximum one can assume that the molecular weight of the copolymer in sample PS-b-PEO 8 is higher.

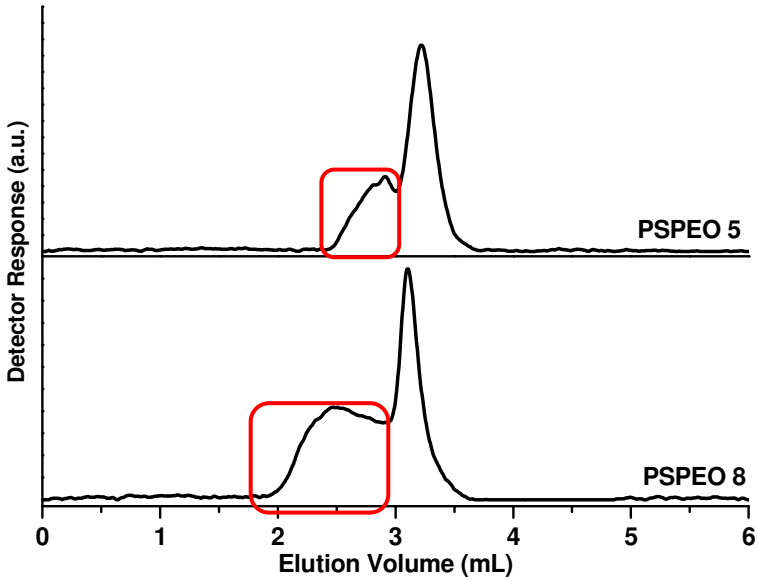

Figure 4.20: PS-b-PEO 5 (M_w of PS 30000 g/mol and M_w of PEO 30000 g/mol) and PS-b-PEO 8 (M_w of PS 109000 g/mol and M_w of PEO 109000 g/mol) run at critical conditions of PS (THF:DMF 18: 82 vol.%).

Table 4.3 shows the V_e for the copolymer peaks and the corresponding molecular weights based on the PEO calibration curve in **Figure 4.13**. These results are then compared to the molecular weight data from the manufacturer. As can be seen the obtained V_e are much higher than what they would be according to the corresponding manufacturer's data. The apparent reason is that for this solvent system the PS block contributes to the retention of the PEO block. It can also be observed that above 50000 g/mol the exclusion limit is reached.

Table 4.3: Comparison of the obtained V_e at the critical conditions of PS (THF:DMF 18:82 vol.%) and their corresponding M_p (according to PEO calibration curve from Figure 4.13) with the manufacturer's M_p.

	Copolymer Peak	PEO calib.	Manuf. data
	V_e (mL)	M_p (g/mol)	M_p (g/mol)
PS-b-PEO 1	2.80	830	3170
PS-b-PEO 2	2.77	1130	3960
PS-b-PEO 3	2.76	1280	3150
PS-b-PEO 4	2.42	7120	29000
PS-b-PEO 5	2.91	-	30000
PS-b-PEO 6	2.43	6970	61500
PS-b-PEO 7	2.31	10860	104000
PS-b-PEO 8	2.48	5920	109000

4.3.4. Preparative fractionation and analysis of fractions

Samples 7 and 8 were selected to be fractionated for further detailed analysis. Both samples were run at the critical conditions of PS and the fractions were collected as indicated in **Figure 4.22** and **Figure 4.21**.

FTIR spectra were collected for the above mentioned fractions for PS-b-PEO 7 and 8 and those are shown in **Figure 4.23** and **Figure 4.24** respectively. When comparing the spectra of the two fractions of PS-b-PEO 7 (**Figure 4.23**) with each other, it can be observed that both fractions contain PS and PEO. From the quantitative FTIR results it was found that fraction 2 (F2) has a much lower PS content (approx. 28 wt.%) compared to fraction 1 (F1) (approx. 48 wt.%) therefore it can be assumed that F1 is block copolymer and F2 mostly PEO homopolymer but it can also be just the chemical heterogeneity of the block copolymer.

Comparing the spectra of the fractions of PS-b-PEO 8 (**Figure 4.24**) with each other it can be seen that F1 contains PS and PEO while F2 contains mainly PS as expected. The PEO that is detected in F2 is most probably due to some fractionation inaccuracy. From the quantitative FTIR results it was found that F1 has approximately 33 wt.% PS content in the copolymer. In other words 1/3 (approximately 11 wt.%) of the overall PS present in the original PS-b-PEO 8 is found in the real block copolymer.

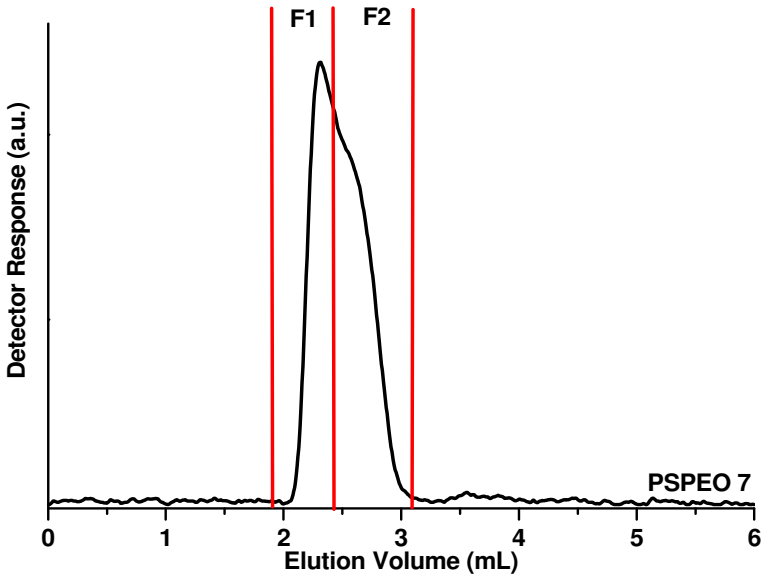

Figure 4.21: Fractionation limits for PS-b-PEO 7 at critical conditions of PS (THF:DMF 18:82 vol.%).

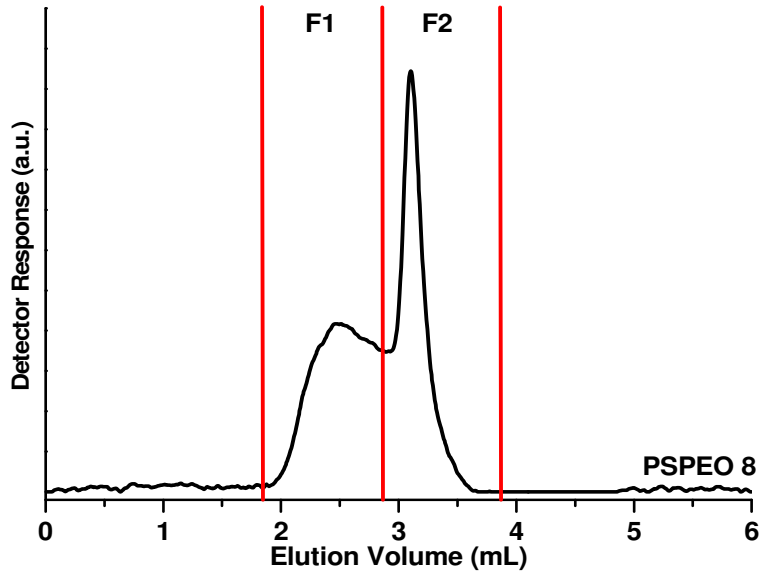

Figure 4.22: Fractionation limits for PS-b-PEO 8 at critical conditions of PS (THF:DMF 18:82 vol.%).

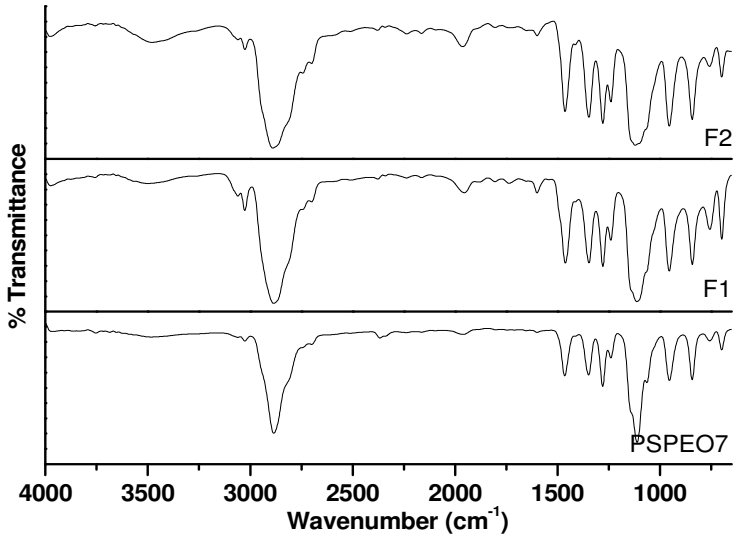

Figure 4.23: FTIR spectra for the fractions of PS-b-PEO 7 fractionated at the critical conditions of PS (THF:DMF 18:82 vol.%)

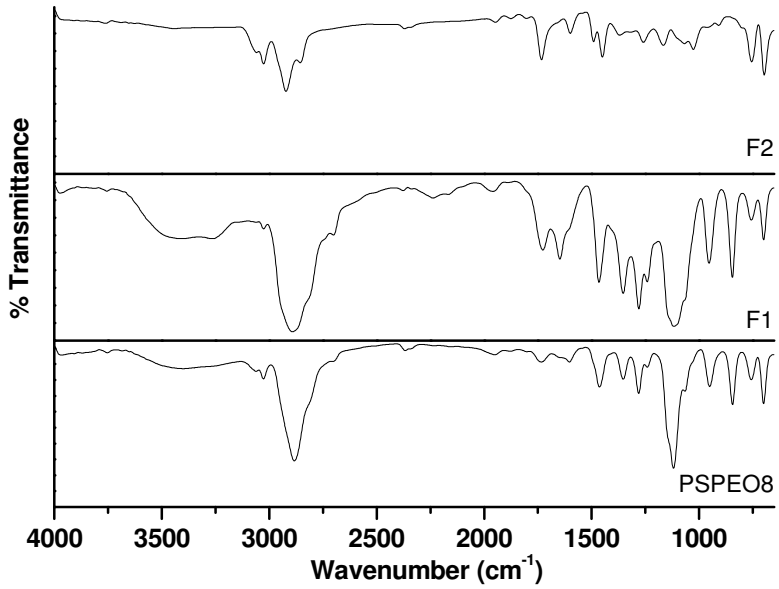

Figure 4.24: FTIR spectra for the fractions of PS-b-PEO 8 fractionated at the critical conditions of PS (THF:DMF 18:82 vol.%).

Fraction 1 of PS-b-PEO 8 fractionated at critical conditions of PS (fraction name: PSPEO8-F1-LCCCofPS) was then selected and analysed at critical conditions of PEO (DMF:THF 4:96 vol.%). The chromatogram obtained from the analysis of PSPEO8-F1-LCCCofPS is shown in **Figure 4.25**. When comparing PSPEO8-F1-LCCCofPS with the unfractionated PS-

b-PEO 8 it can be observed that both chromatograms look very similar. The second eluting peak appears at a V_e that corresponds to the elution of PEO homopolymer, compare **Figure 4.35**. Thus it can be said that the analysed fraction contains block copolymer as well as PEO homopolymer.

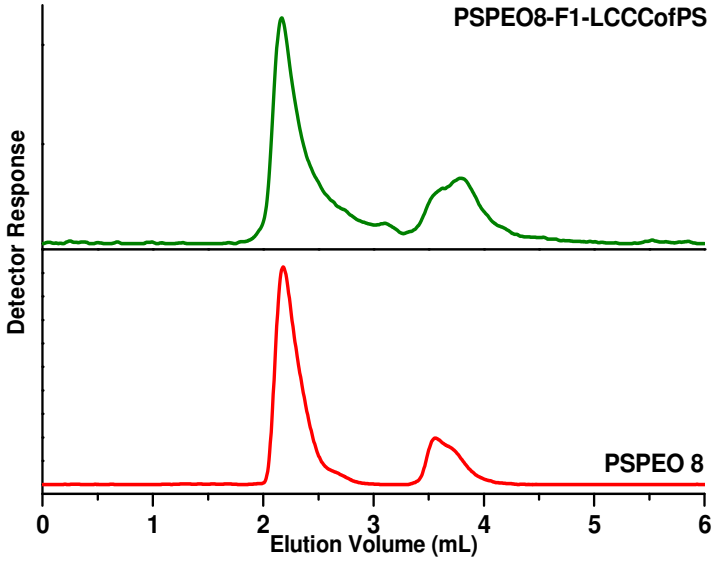

Figure 4.25: PS-b-PEO 8 and PSPEO8-F1-LCCCofPS run at the critical conditions of PEO (DMF:THF 4:96 vol.%).

4.3.5. Two-dimensional chromatography

The analysis of the copolymers using LCCC of PS was followed by two-dimensional liquid chromatography (2D-LC) analysis of the copolymers. Critical conditions of PS were used in the first dimension and SEC using DMF as the eluent for the second dimension. DMF was used as the eluent for the second dimension because in pure THF the block copolymer samples could not be completely dissolved, not even upon heating. The PS calibration curve (**Figure 3.1**) was applied to the obtained 2D data. For comparison the original data with an elution volume axis are shown in **Figure 4.31**. The resulting 2D plots are shown below. When looking at the plot, for example of PS-b-PEO 1 (**Figure 4.26**), the y-axis shows the LCCC of PS separation (separation according to chemical composition) and the x-axis shows the SEC separation (separation according to size) to which the PS calibration curve was applied in order to obtain a molar mass scale. The black dots are just defects, but where difficult to remove without compromising the actual 2D-plots (encircled) of the samples.

Comparing PS-b-PEO 1 to PS-b-PEO 4 (**Figure 4.26** to **Figure 4.29**) an increase in molecular weight can be observed. The amount of PS homopolymer which was observed for PS-b-PEO 1 and PS-b-PEO 2 in the one dimensional chromatographic analysis (**Figure 4.15**) is too small compared to the copolymer and, therefore, it could not be seen in the 2D plots. For PS-b-PEO 5 and PS-b-PEO 8 (**Figure 4.30** and **Figure 4.33**) copolymer and homopolymer peaks can be seen. The molecular weight for the PS homopolymers in those two samples is in an increasing order as it is the case for the copolymer in those two samples. For PS-b-PEO 6 and PS-b-PEO 7 the shoulders which were observed in **Figure 4.18** can clearly be seen in their 2D plots (**Figure 4.32** and **Figure 4.33** respectively).For PS-b-PEO 7 it can clearly be seen that there are two molecular weight distributions while for PS-b-PEO 6 only broad molecular weight distribution is observed. Comparing the two samples' molecular weights a slight increase from PS-b-PEO 6 to PS-b-PEO 7 can be seen.

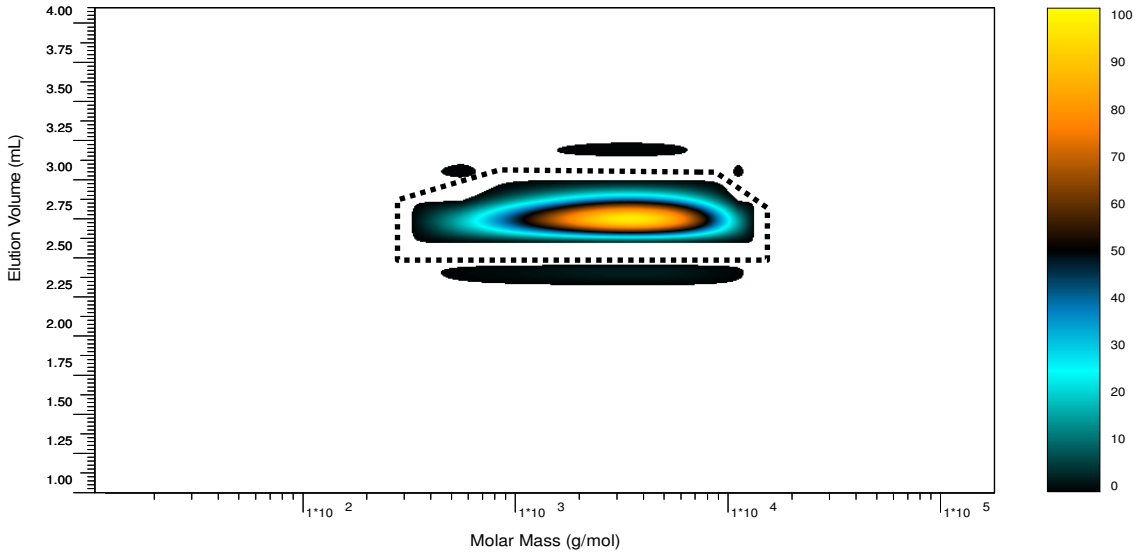

Figure 4.26: PS-b-PEO 1 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nd dimension: SEC with DMF as eluent. PS calibration was applied.

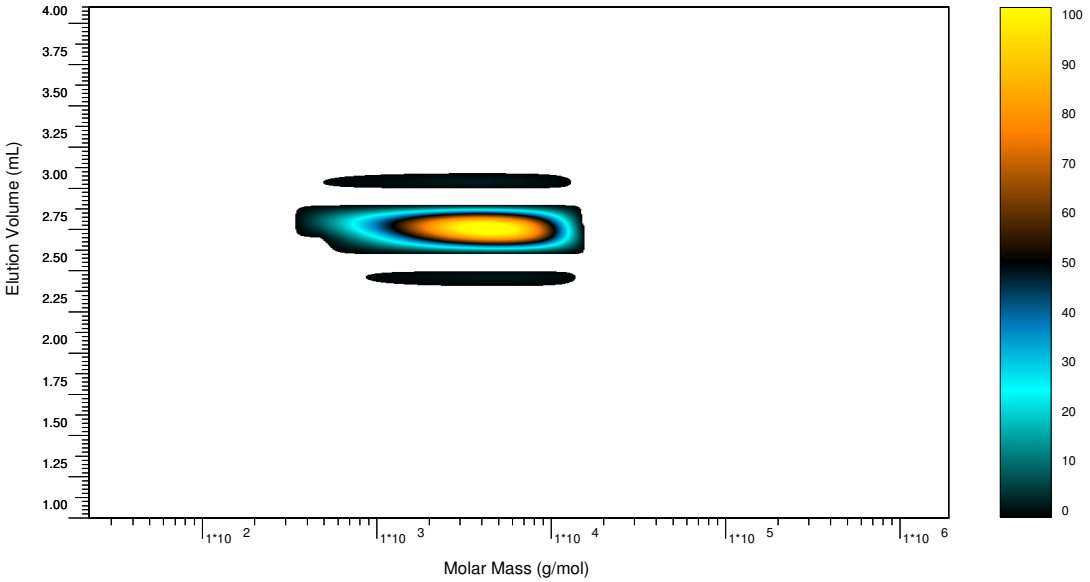

Figure 4.27: PS-b-PEO 2 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nddimension: SEC with DMF as eluent. PS calibration was applied.

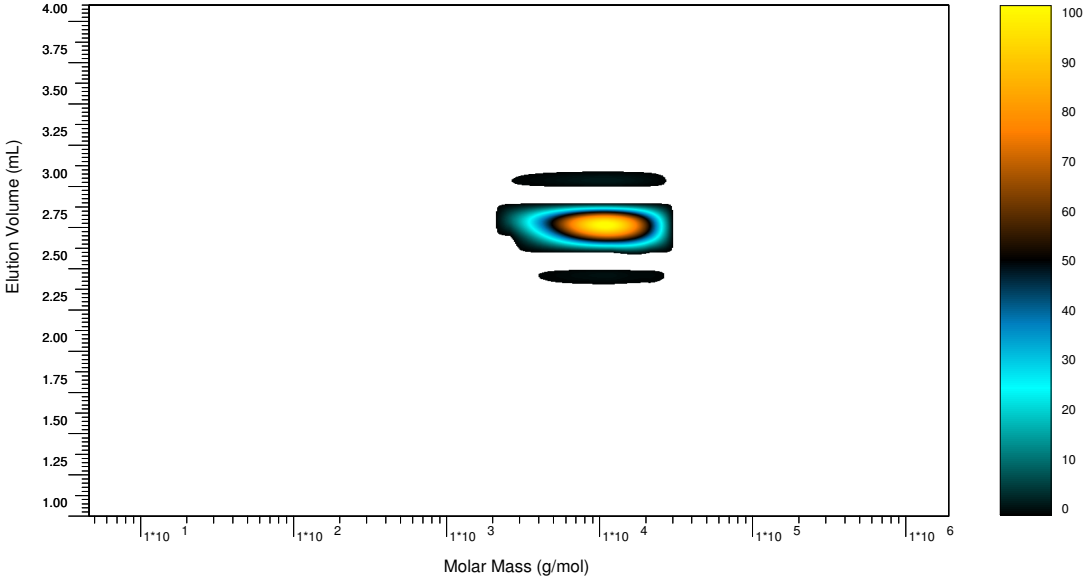

Figure 4.28: PS-b-PEO 3 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nddimension: SEC with DMF as eluent. PS calibration was applied.

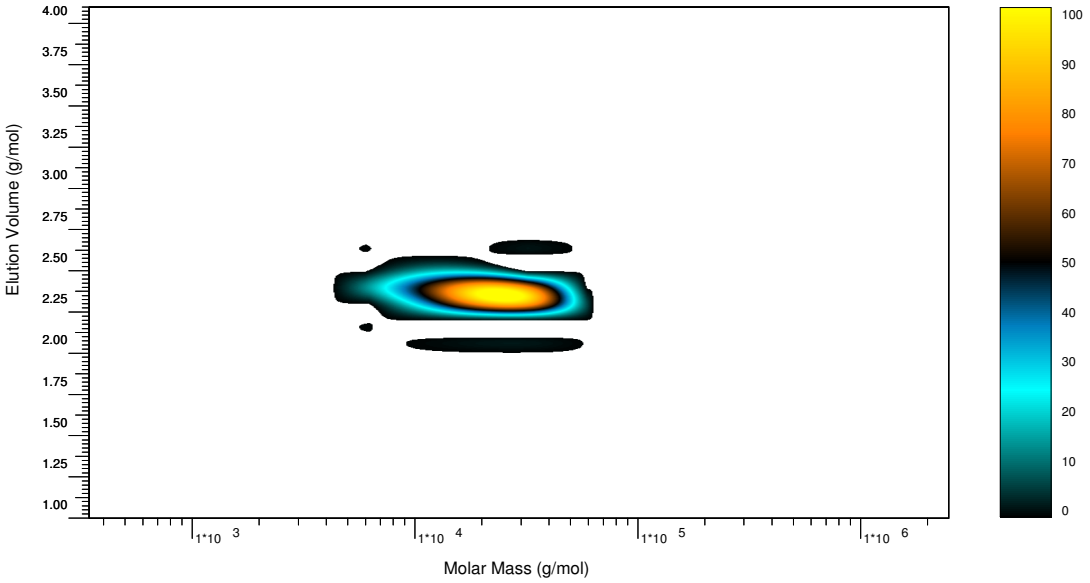

Figure 4.29: PS-b-PEO 4 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nd dimension: SEC with DMF as eluent. PS calibration was applied.

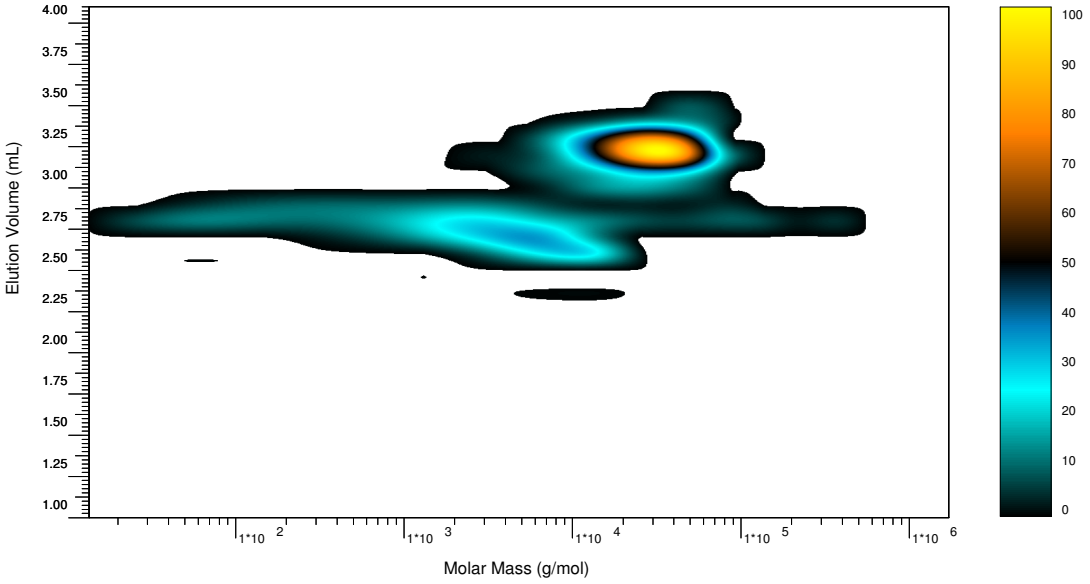

Figure 4.30: PS-b-PEO 5 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nd dimension: SEC with DMF as eluent. PS calibration was applied.

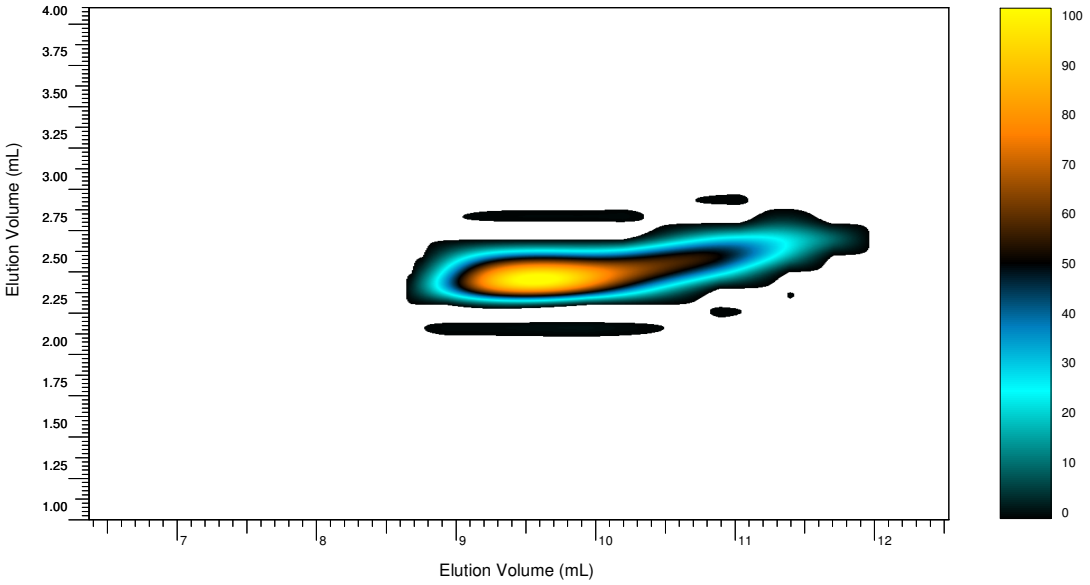

Figure 4.31: PS-b-PEO 6 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nd dimension: SEC with DMF as eluent.

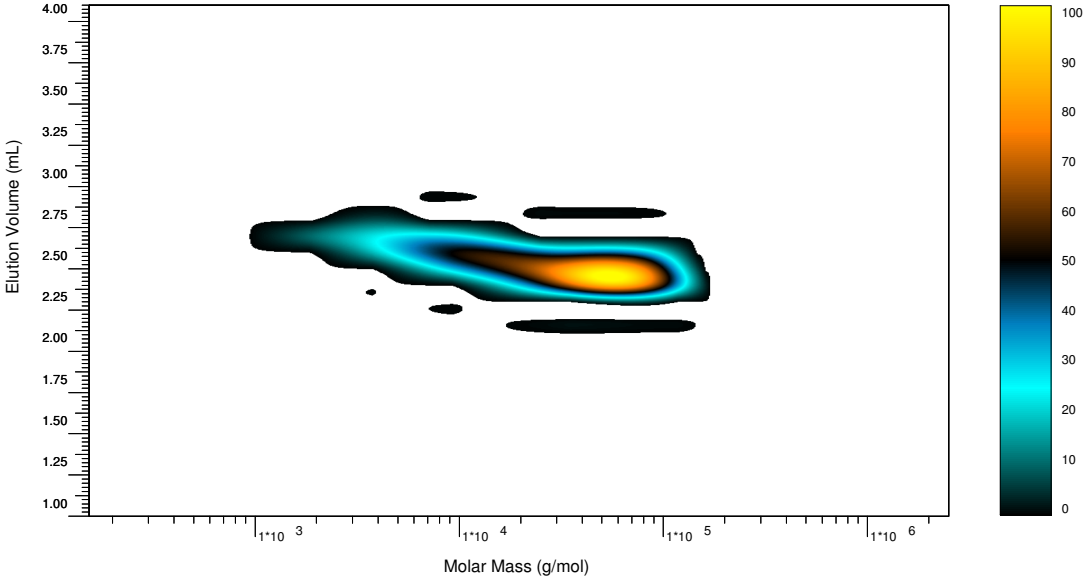

Figure 4.32: PS-b-PEO 6 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nd dimension: SEC with DMF as eluent. PS calibration was applied.

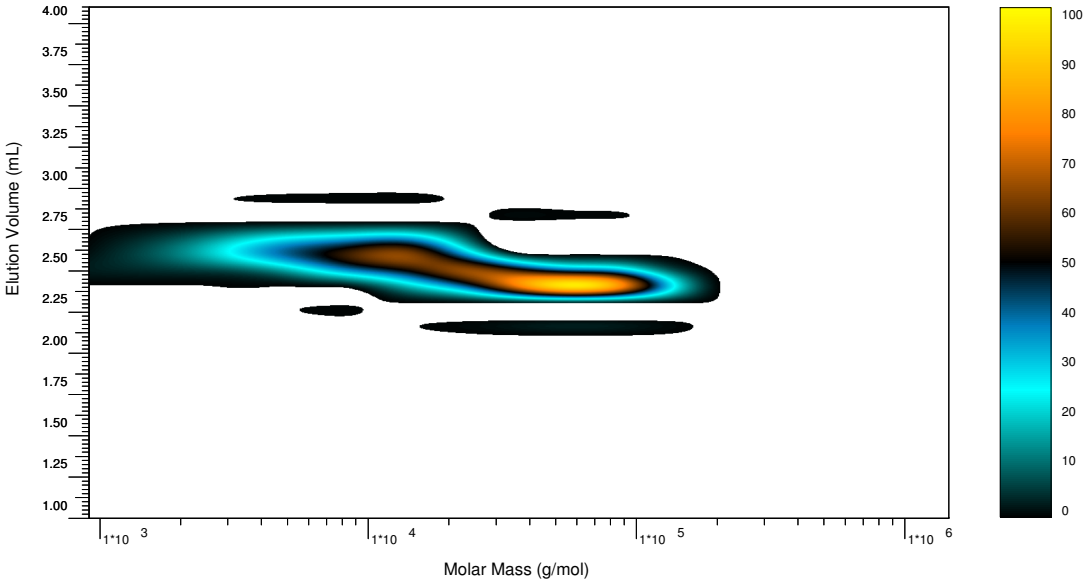

Figure 4.33: PS-b-PEO 7 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nd dimension: SEC with DMF as eluent. PS calibration was applied.

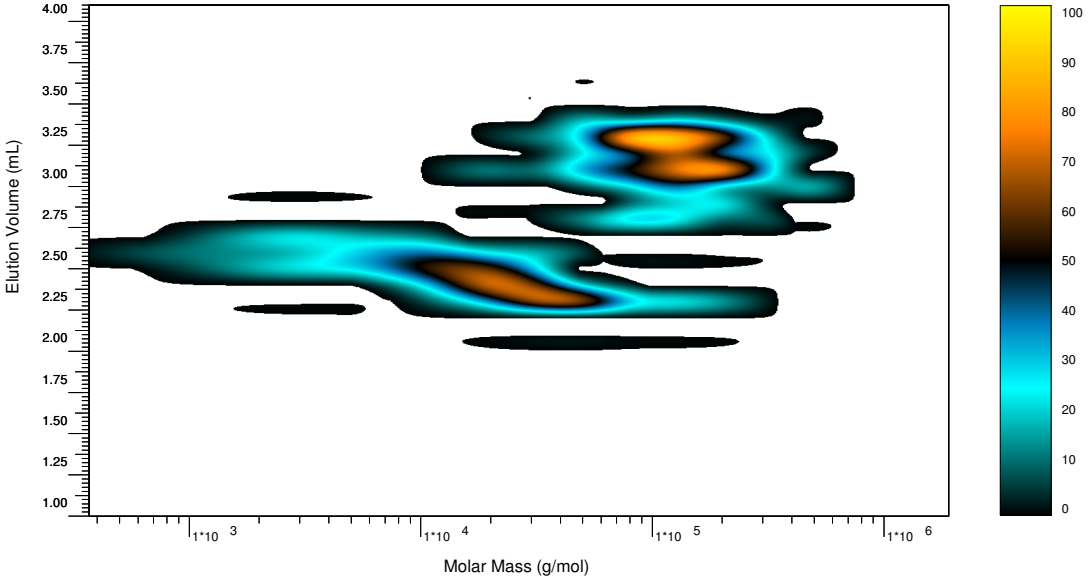

Figure 4.34: PS-b-PEO 8 2D-LC plot. 1st dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2nd dimension: SEC with DMF as eluent. PS calibration was applied.

The molecular weight results for the PS homopolymers as well as the copolymers from the 2D plot for the eight samples are summarized in **Table 4.4**. When comparing the determined PS homopolymer molecular weight results with those from the manufacturer it can be noted that they are quite close. The molecular weight results for the copolymer fractions that might

contain PEO homopolymer are quite diverse. For rather homogeneous 2D plots indicating rather homogeneous samples there is a quite good agreement between the 2D results and the manufacturer's data. This can be seen for samples 1, 2, 4 and 6. For the more heterogeneous samples such as samples 5, 7 and 8 a reliable quantification of the block copolymer molecular weight is not possible. It can be suspected that due to the presence of large amounts of PEO homopolymer which is coeluting with the copolymer, a proper molecular weight analysis cannot be conducted.

Table 4.4: Determined M_p for the PS homopolymer and the copolymer fractions with the help of the 2D-LC (1^{st} dimension: critical conditions of PS (THF:DMF 18:82 vol.%) 2^{nd} dimension: SEC with DMF as eluent). PS calibration curve was used.

	V_e of PS homopolymer (mL)	**M_p of PS homopolymer (g/mol)**	**V_e of block copolymer & PEO homo. (mL)**	**M_p of block copolymer & PEO homo. (g/mol)**	**M_w PS (g/mol)**
PS-b-PEO 1	-	-		3700	M_n = 1500
PS-b-PEO 2	-	-	11.33	5000	M_n = 1500
PS-b-PEO 3	-	-	10.81	11200	3940
PS-b-PEO 4	-	-	10.20	29000	2930
PS-b-PEO 5	10.03	36000	11.01	8100	30000
PS-b-PEO 6	-	-	9.56	64600	30000
PS-b-PEO 7	-	-	10.68 and 9.51	14400 and 67800	30000
PS-b-PEO 8	8.98	114400	10.05	35200	109000

To quantify the PS homopolymer a calibration curve for the ELSD was established (see **Figure 4.35**) as described in **Section 3.2.2**. For this calibration curve PS calibration standards were used. It can be seen that the PS homopolymer with the smallest peak area per injected mass is M_p of 580 g/mol. This is because the smallest oligomers might undergo partial evaporation with the solvent due to the elevated ELSD temperature settings. The maximum peak area per injected mass is obtained for 29510 g/mol PS calibration standard. At lower injected masses the high molar mass calibration standards behave similarly indicating that

there is no pronounced effect of the molecular weight on the detector sensitivity. At higher injected masses the relative peak area decreases with increasing molecular weight. This might be due to the fact that droplet formation changes with molecular weight or that some material is adsorbed on the stationary phase. The amount of PS homopolymer present in the copolymer samples can be calculated with the help of this calibration curve. The results will be shown in the last section of this chapter.

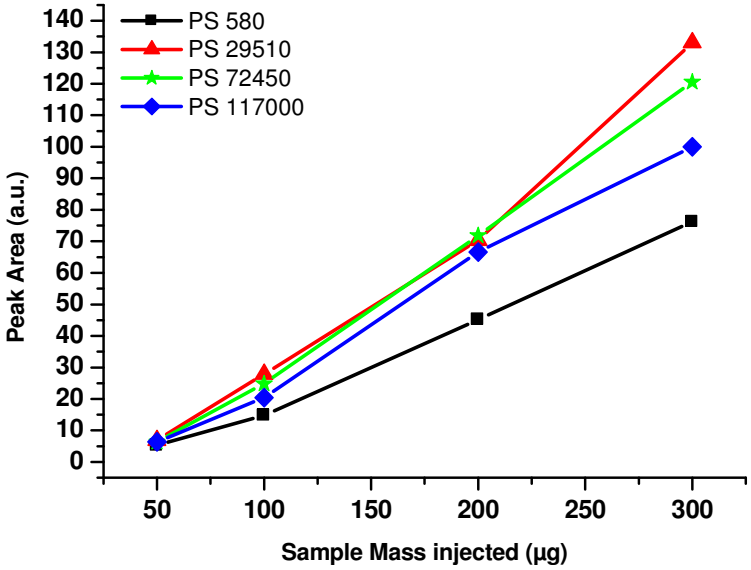

Figure 4.35: ELSD calibration curves for PS with different molecular weights using 1D LCCC of PS. ELSD conditions are 180ºC for evaporation and 80ºC for nebulisation at a N_2 gas flow rate of 1.5 SLM.

4.4. Critical conditions of PEO with DMF-THF

For the critical conditions of PEO, DMF (good solvent, which promotes desorption of sample from silica based stationary phase) and THF (poor solvent, which promotes adsorption of sample to a silica based stationary phase) was used as mobile phase. From the work of Berek[5]the approximate ratio of the two solvents at the critical conditions of PEO was known. Different ratios of DMF:THF from (50:50 vol.%) to (0:100 vol.%) were pre-mixed. Pre-selected PEO calibration standards where dissolved in the different pre-mixed solvent compositions.

The resulting log (M_p) vs. V_e plots using the different solvent compositions are shown in **Figure 4.36**. As can be seen the PEO calibration standards elute in the SEC mode (lower V_e) when using a DMF:THF composition of 50:50 vol.%. When using 100 vol.% THF the PEO calibration standards elute in LAC mode (higher V_e).The solvent composition of 4:96 vol.% (DMF:THF) was the closest to the critical conditions that could be obtained.

As can be observed, up to a M_p of 114000 g/mol the calibration standards elute at nearly the same V_e. The PEO calibration standards used are from two manufacturers and, therefore, might slightly vary in chemical composition due to different synthetic procedures (see **Table** 3.2). The slight variation in critical elution volume for the PEO standard 23600 g/mol might indicate this situation.

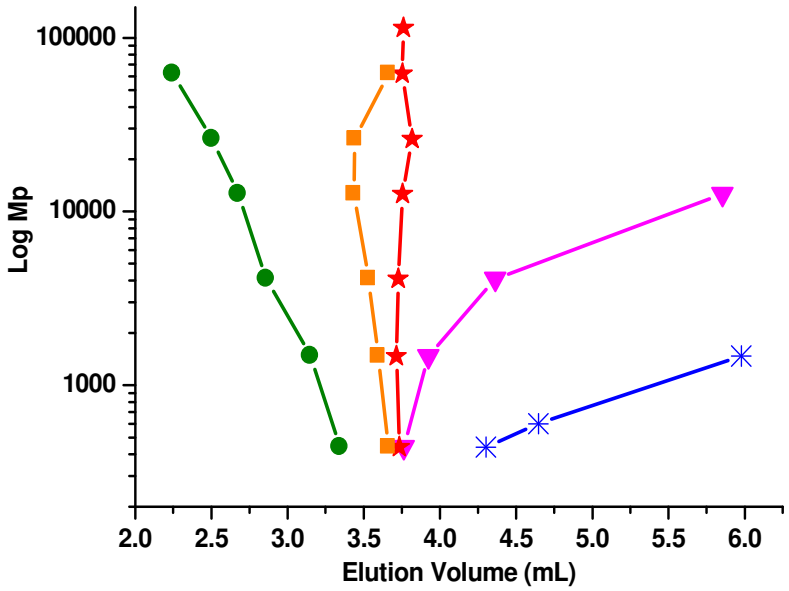

Figure 4.36: Plots of log M_p vs. V_e of PEO at different THF:DMF ratios. ✳ =(0:100, ▼ = 2:98, ★ = 4:96, and ■ = 5:95● = 50:50 vol.%. Column used: 300Å Nucleosil Si, 4.6 x 250 mm at 29.7ºC

In **Figure 4.37** the PS calibration curve is presented that was obtained at chromatographic conditions corresponding to the critical point of PEO. As can be seen, proper resolution is achieved up to a molecular weight of about 117000 g/mol for PS. This calibration should allow determining the block lengths of the PS blocks in the block copolymers.

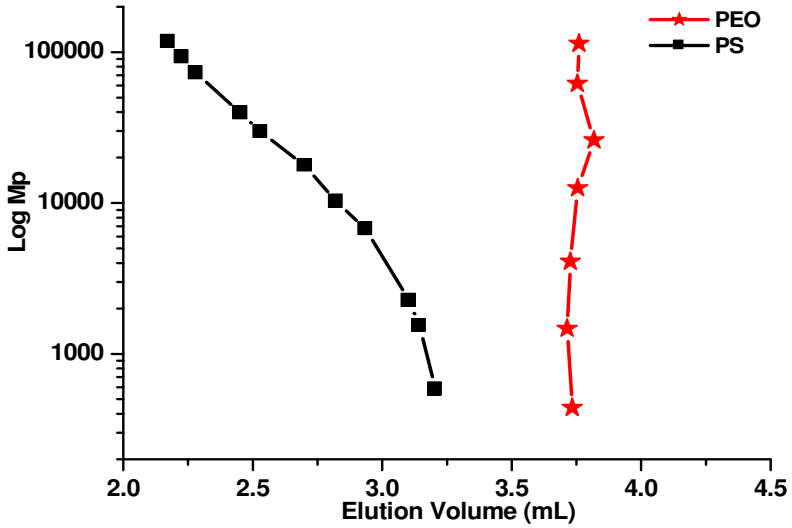

Figure 4.37: Plots of log M_p vs. V_e of PEO and PS at critical conditions of PEO with DMF:THF at a ratio of 4:96 vol.%. Column: 300Å Nucleosil Si, 4.6 x 250 mm at 29.7°C.

4.4.1. LCCC method development

Two different blends of PS and PEO with similar molecular weights were investigated in the established critical conditions. The two blends (**Figure 4.38**) were baseline separated. The black box shows PEO with different molecular weights eluting at the same V_e, indicating that they elute irrespective of their molecular weight at critical conditions. The PS with different molecular weights elute in the order of decreasing molecular weights as dotted arrow indicates.

When looking at the chromatograms of PS-b-PEO 1 and PS-b-PEO 2 in **Figure 4.39** it is observed once again, that there is not much difference between the two samples. From the manufacturer's data it is known that both samples should have the same PS block length. The main peak is copolymer showing the presence of some PS homopolymer (shoulder encircled) which is confirmed by the chromatogram of the same sample in **Figure 4.15** (PS homopolymer peak is encircled). In **Section 4.3.3** it was not yet clear if there is some PEO homopolymer present or not. At this stage, if there would be some PEO homopolymer present a peak would be visible in the area where the PEO calibration standards elute, which is approximately 3.75-3.82 mL (area indicated by dotted box).In that same section, it was also

mentioned that the homopolymers eluted at a slightly lower V_e due to the possible presence of different end groups. The same is valid for PEO homopolymer. If the actual amount of PEO homopolymer compared to the amount of the copolymer is much less and if it has different end groups compared to the calibration standards, the peak might be obscured by the copolymer peak, thus not even a shoulder would be visible. Therefore it is assumed that there is no PEO homopolymer present in these samples.

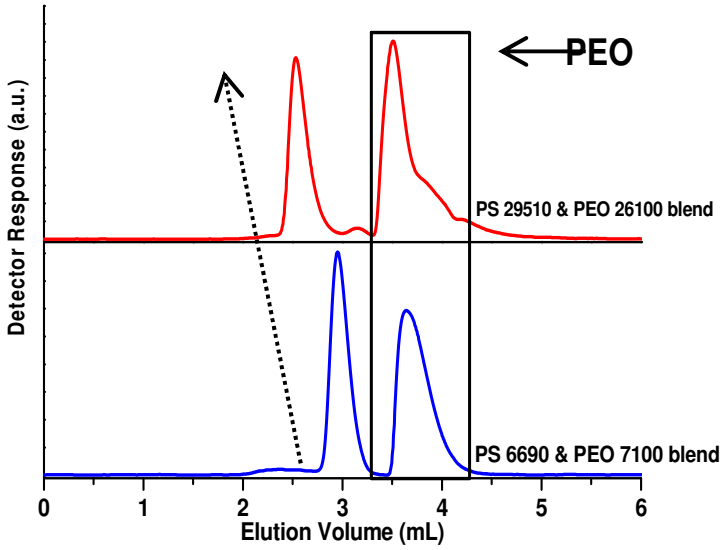

Figure 4.38: Blends of PS and PEO calibration standards run at the critical conditions of PEO (DMF:THF 4:96 vol.%).

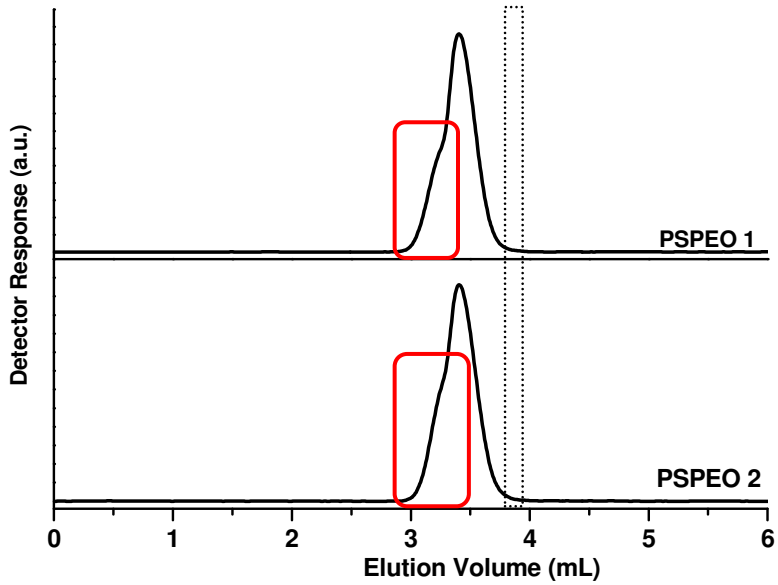

Figure 4.39: PS-b-PEO 1 (M_w of PS 1500 g/mol and M_w of PEO 3170 g/mol) and PS-b-PEO 2 (M_w of PS 1500 g/mol and M_w of PEO 3960 g/mol) run at critical conditions of PEO (DMF:THF 4:96 vol.%).

Figure 4.40 displays samples PS-b-PEO 3 and PS-b-PEO 4. PS-b-PEO 3 shows the presence of copolymer while PEO homopolymer was not detected. PS-b-PEO 4 elutes at a significantly higher V_e than PS-b-PEO 3, which indicates that it has lower molecular weight PS blocks compared to PS-b-PEO 3. PS-b-PEO 4 shows two non-separated peaks. Knowing that the samples might have different end groups compared to the calibration standards and thus might elute at slight lower V_e than the PEO calibration standards, the encircled peak might be PEO homopolymer. The other peak shows the presence of block copolymer.

PS-b-PEO 6 and PS-b-PEO 7 (**Figure 4.41**) both show two well separated peaks. The encircled peaks are due to PEO homopolymer and the others are due to copolymers. For these two samples it is clear that the latter peaks are only due to the copolymer since from **Figure 4.18** it was found that these samples do not include any PS homopolymer. And now it can be said for sure that the peak with the shoulder of PS-b-PEO 7 in **Figure 4.18** was due to the block copolymer and PEO homopolymer. When comparing the V_e of the copolymers of each of the two samples, it can be seen that the copolymer elutes at the same elution volume, thus indicating that the blocks of the two samples have the same molecular weight.

In **Figure 4.42** the chromatograms for PS-b-PEO 5 and 8 are presented. For both these samples baseline separation was obtained resulting in PEO homopolymer as well as copolymer peak. When comparing the copolymer fraction of the two samples it can be observed that the molecular weight of PS-b-PEO 5 is lower than for PS-b-PEO 8.

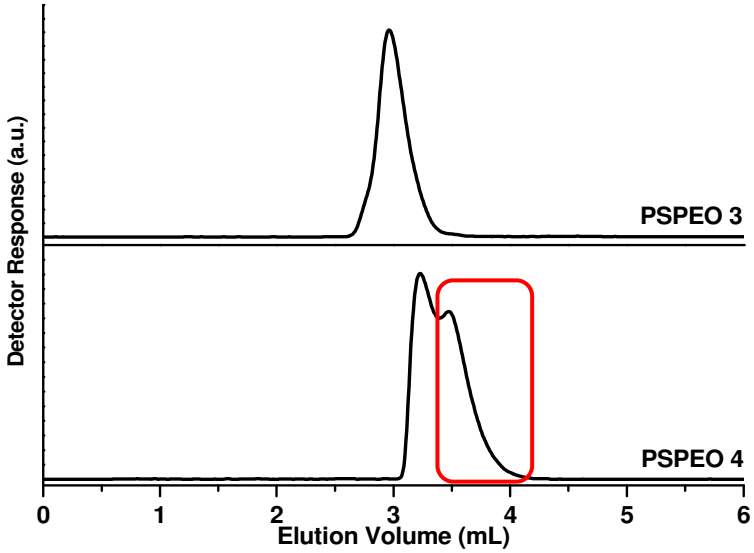

Figure 4.40: PS-b-PEO 3 (M_w of PS 3940 g/mol and M_w of PEO 3150 g/mol) and PS-b-PEO 4 (M_w of PS 2930 g/mol and M_w of PEO 29000 g/mol) run at critical conditions of PEO (DMF:THF 4:96 vol.%).

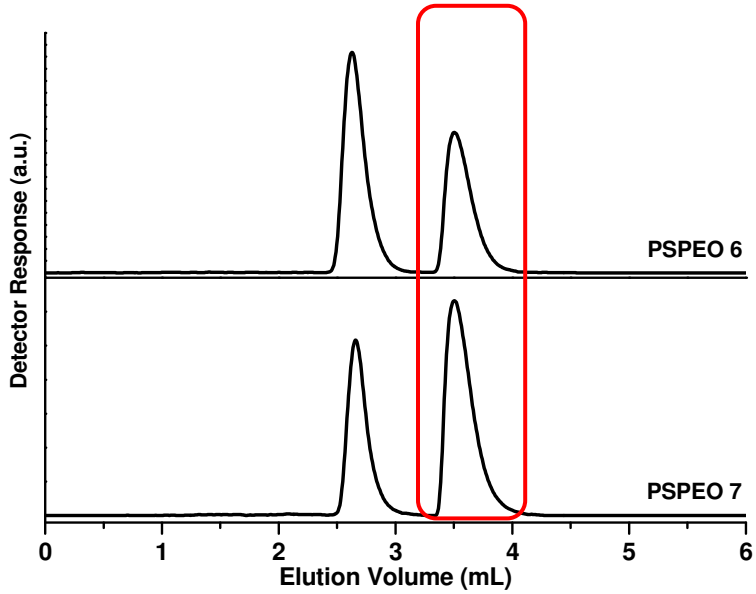

Figure 4.41: PS-b-PEO 6 (M_w of PS 30000 g/mol and M_w of PEO 61500 g/mol) and PS-b-PEO 7 (M_w of PS 30000 g/mol and M_w of PEO 104000 g/mol) run at critical conditions of PEO (DMF:THF 4:96 vol.%).

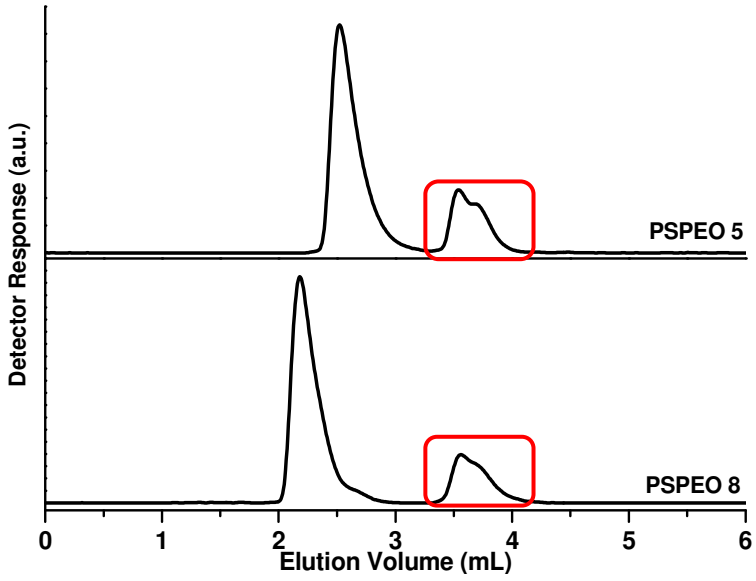

Figure 4.42: PS-b-PEO 5 (M_w of PS 30000 g/mol and M_w of PEO 30000 g/mol) and PS-b-PEO 8 (M_w of PS 109000 g/mol and M_w of PEO 109000 g/mol) run at critical conditions of PEO (DMF:THF 4:96 vol.%).

Table 4.5 shows the V_e of the copolymer peaks and what their molecular weight would be according to the PS calibration curve in **Figure 4.37**. These results are then compared to the molecular weight data from the manufacturer. It can be seen that, for the lower molecular weight samples 1-4 the elution volumes are much higher than expected. For the higher molecular weight samples there is a fairly good agreement between the experimental and the expected molecular weights. It is not clear at present why this is the case, however, further studies will be conducted to investigate this phenomenon.

Table 4.5: Comparison of the obtained V_e at the critical conditions of PEO (DMF:THF 4:96 vol.%) and their corresponding M_p (according to PS calibration curve from Figure 4.37) with the manufacturer's M_p.

	Copolymer Peak	PS calib.	Manuf. data
	V_e (mL)	M_p (g/mol)	M_w (g/mol)
PS-b-PEO 1	3.40	-	1500
PS-b-PEO 2	3.41	-	1500
PS-b-PEO 3	2.96	6400	3940
PS-b-PEO 4	3.24/3.48	-/-	2930
PS-b-PEO 5	2.54	29000	30000
PS-b-PEO 6	2.63	22700	30000
PS-b-PEO 7	2.64	22000	30000
PS-b-PEO 8	2.18	114000	109000

4.4.2. Preparative fractionation and analysis of fractions by FTIR

Samples 5, 7 and 8 were selected to be fractionated at these critical conditions for further detailed analysis. The fractionation limits for PS-b-PEO 8 and for PS-b-PEO 5 and 7 are indicated in **Figure 4.44** and **Figure 4.43** respectively.

FTIR spectra were collected for the above mentioned fraction for PS-b-PEO 5, 7 and 8 and the spectra are shown in **Figure 4.45** to **Figure 4.47** respectively. When comparing the spectra of the three fraction of PS-b-PEO 5 (**Figure 4.45**) with each other, it can be observed that F1 mostly contains PS while the other two fractions only have PEO. From the quantitative FTIR

results it was found that F1 has approximately 92 wt.% of PS present confirming what was observed in the spectra of F1. What can be gathered from these FTIR results at this stage for PS-b-PEO 5 is that it contains rather small amounts of block copolymer and rather large amounts of PS and PEO homopolymers.

The spectrum for F1 of PS-b-PEO 7 (**Figure 4.46**) shows that this fraction contains PS and PEO while F2 only has PEO. From the quantitative FTIR results it was determined that F1 has approximately 68 wt.% PS. Therefore it can be assumed that PS-b-PEO 7 includes block copolymer PEO homopolymer and no PS homopolymer.

Comparing the spectra of the fractions of PS-b-PEO 8 (**Figure 4.47**) with each other it can be observed that F1 contains PS and PEO while F2 and F3 shows only the presence of PEO. With the help of quantitative FTIR it was found that F1 has approximately 96 wt.% of PS.

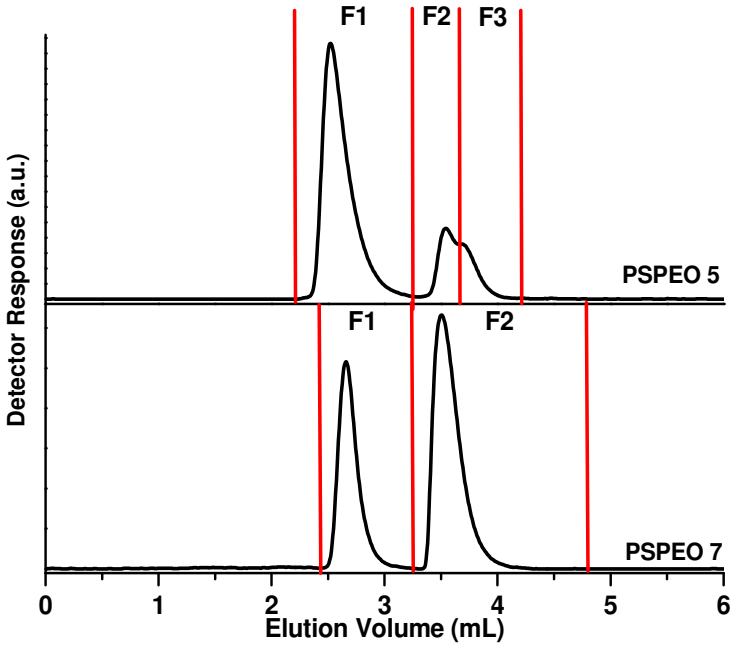

Figure 4.43: Fractionation limits for PS-b-PEO 5 and PS-b-PEO 7 at critical conditions of PEO (DMF:THF 4:96 vol.%).

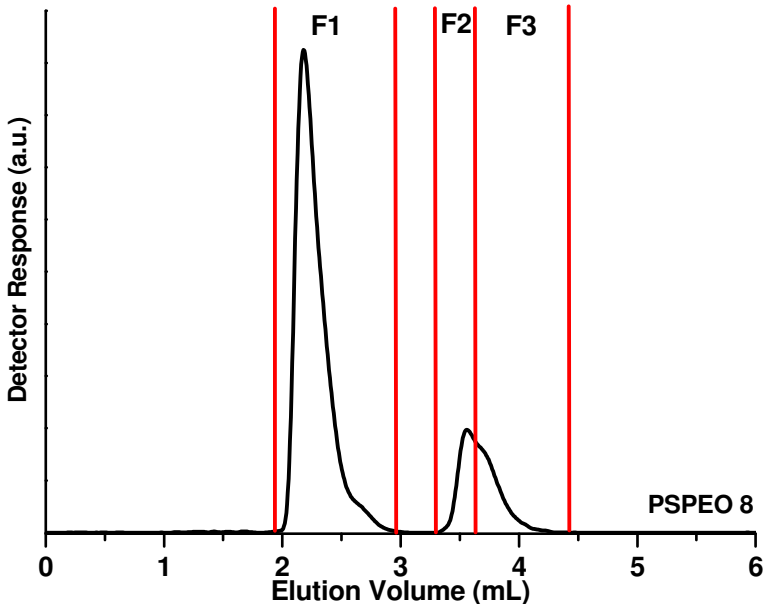

Figure 4.44: Fractionation limits for PS-b-PEO 8 at critical conditions of PEO (DMF:THF 4:96 vol.%).

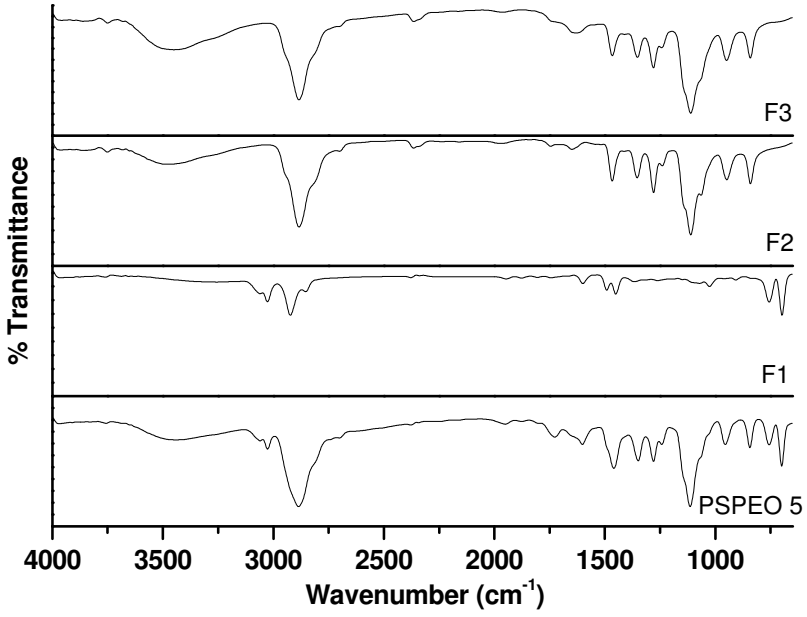

Figure 4.45: FTIR spectra for the fractions of PS-b-PEO 5 fractionated at the critical conditions of PEO (DMF:THF 4:96 vol.%)

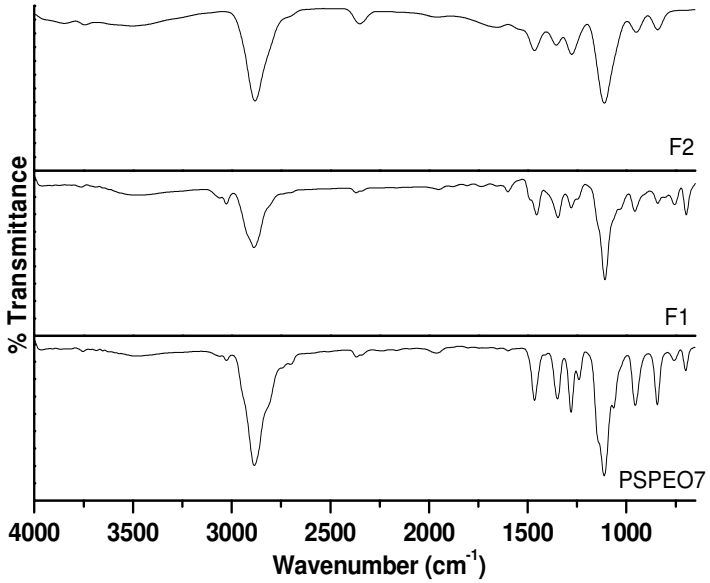

Figure 4.46: FTIR spectra for the fractions of PS-b-PEO 7 fractionated at the critical conditions of PEO (DMF:THF 4:96 vol.%)

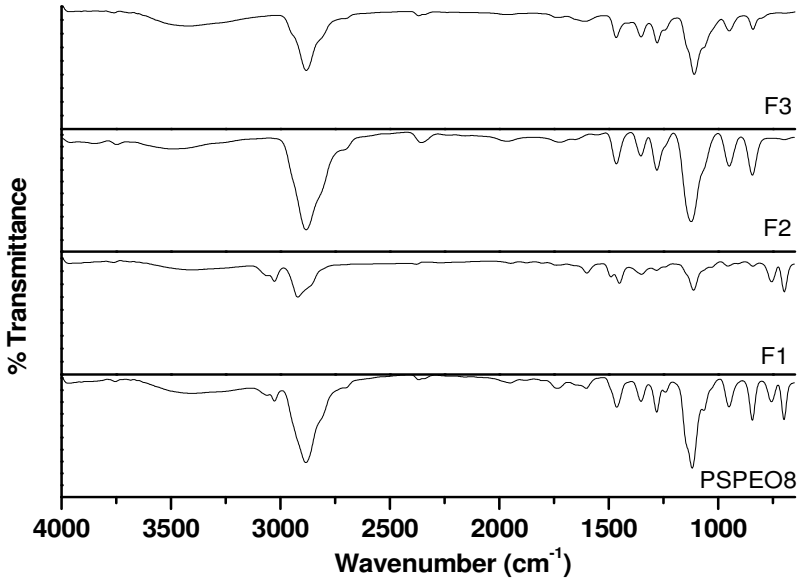

Figure 4.47: FTIR spectra for the fractions of PS-b-PEO 8 fractionated at the critical conditions of PEO (DMF:THF 4:96 vol.%)

Fraction 1 of PS-b-PEO 8 was then selected to be analysed at the critical conditions of PS. The resulting chromatogram is shown in **Figure 4.48**. When comparing PSPEO8-F1-LCCCofPEO (A) with the original PS-b-PEO 8 (B) also analysed at the critical conditions of PS it can be

seen that both look very similar. The encircled peak is due to PS homopolymer and the hump at the lower V_e correlates to block copolymer. Taking the two results of this fraction of PS-b-PEO 8 analysed at both critical conditions of PS and PEO it can be said that PS-b-PEO 8 contains copolymer and PS and PEO homopolymer. Considering the intensities of the different elution peaks it must be concluded that the majority of the sample is composed of PS and PEO homopolymers. The chromatogram in **Figure 4.48**A indicates that the sample contains only small amounts of copolymer.

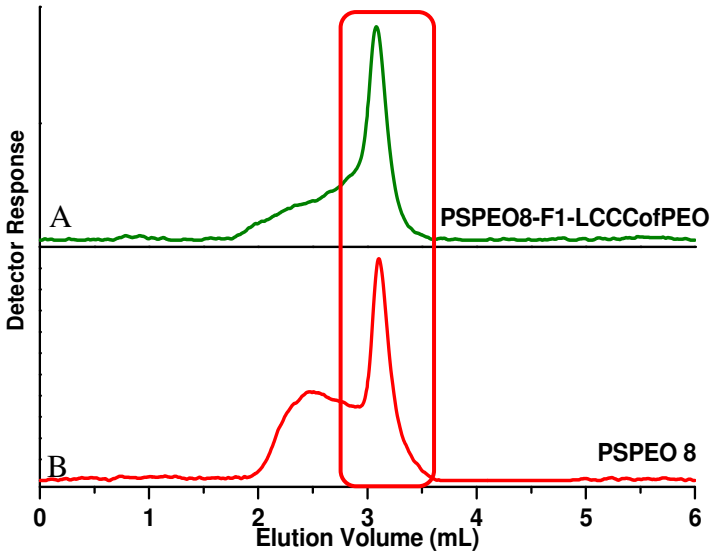

Figure 4.48: PS-b-PEO 8 and PSPEO8-F1-LCCCofPEO run at the critical conditions of PS (DMF:THF 4:96 vol.%).

4.4.3. Two-dimensional chromatography

The analysis of the copolymers using LCCC of PEO was followed by 2D-LC analysis of the copolymers. Critical conditions of PEO were used in the first dimension and SEC using DMF as the mobile phase for the second dimension. The resulting 2D plots are shown below.

When looking at the plots for PS-b-PEO 1 to PS-b-PEO 3 (**Figure 4.49** to **Figure 4.51**) a slight increase in molecular weight can be observed for the latter. For PS-b-PEO 4 and PS-b-PEO 8 (**Figure 4.52** to **Figure 4.57**) copolymer and PEO homopolymer peaks can be seen. The molecular weight for the PEO homopolymers in the latter four samples is quite low

compared to the result of the molecular weight for the copolymers at the critical conditions of PS but a slight increase can be observed. PS-b-PEO 4 has the highest molecular weight PEO homopolymer. **Figure 4.54** is the same 2D plot for PS-b-PEO 6 except not PEO calibration was applied and the original data are supplied.

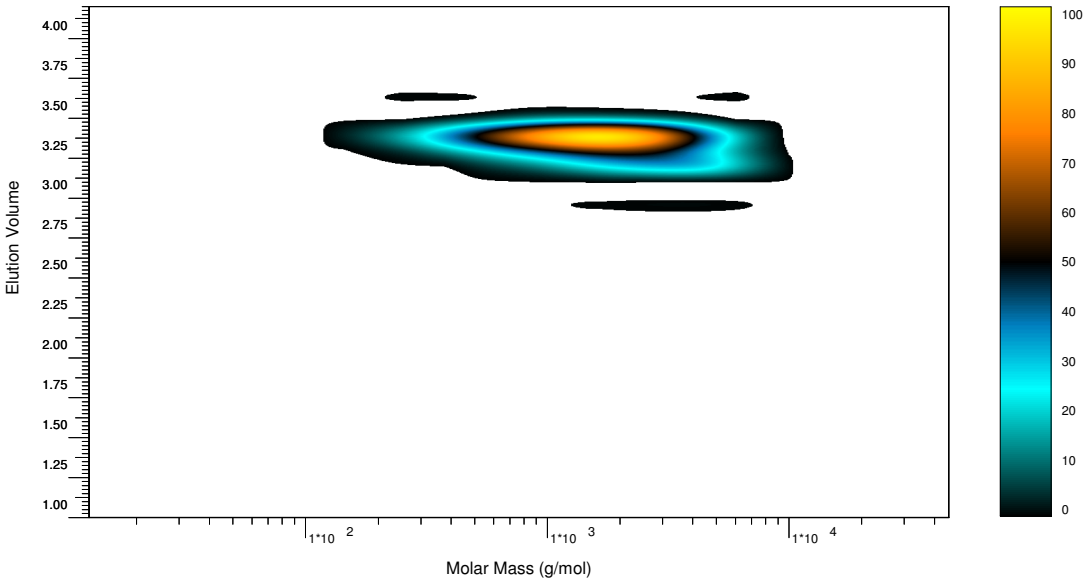

Figure 4.49: PS-b-PEO 1 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nd dimension: SEC with DMF as eluent. PEO calibration was applied.

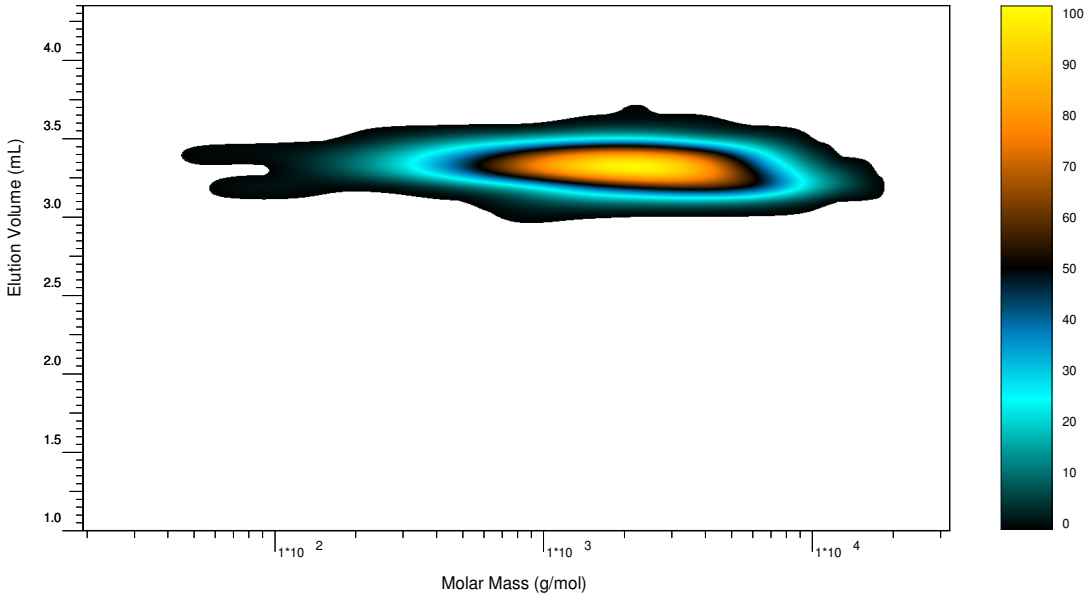

Figure 4.50: PS-b-PEO 2 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nd dimension: SEC with DMF as eluent. PEO calibration was applied.

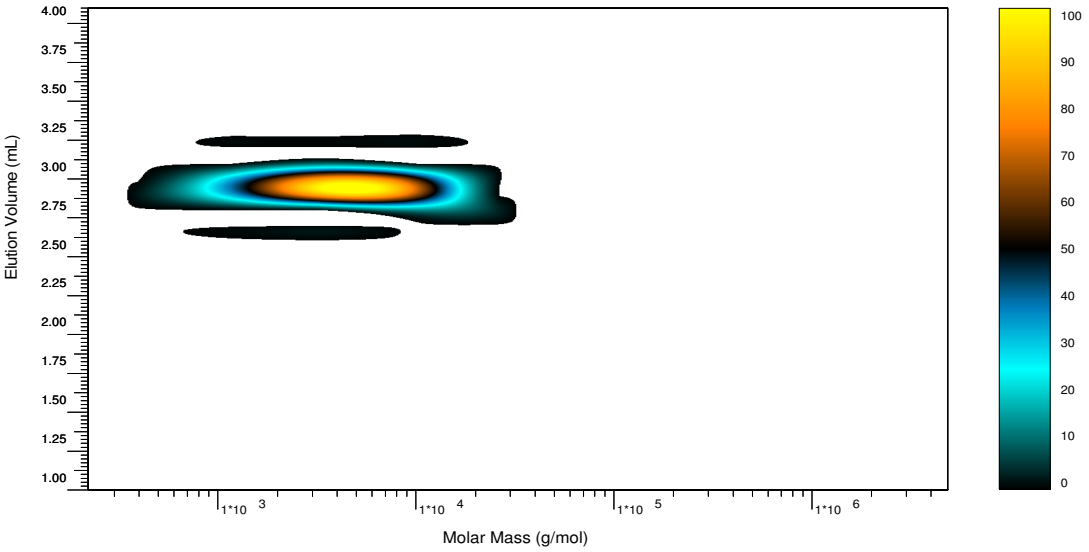

Figure 4.51: PS-b-PEO 3 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nd dimension: SEC with DMF as eluent. PEO calibration was applied.

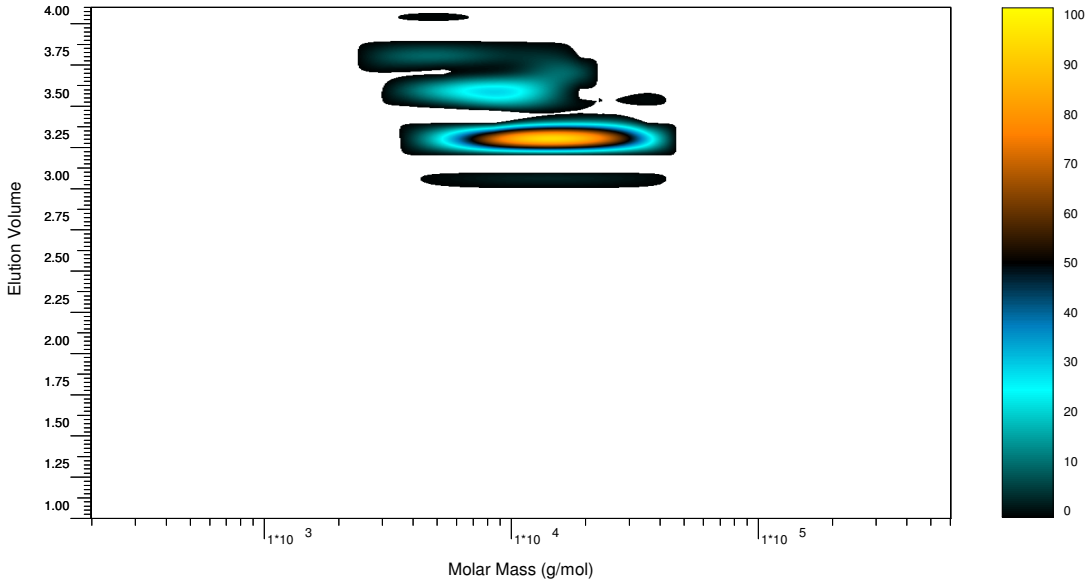

Figure 4.52: PS-b-PEO 4 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nddimension: SEC with DMF as eluent. PEO calibration was applied.

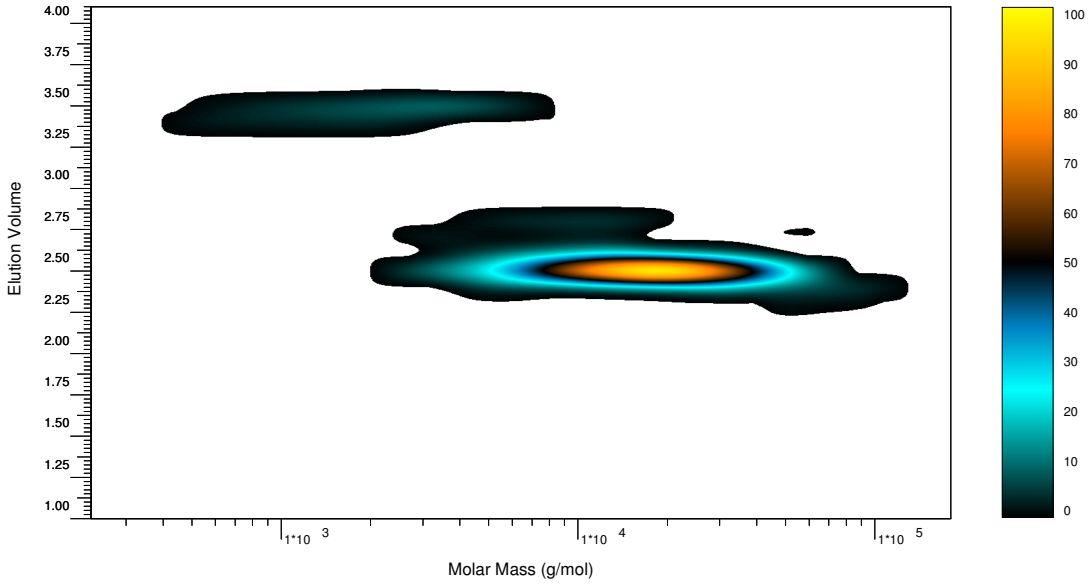

Figure 4.53: PS-b-PEO 5 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nddimension: SEC with DMF as eluent. PEO calibration was applied.

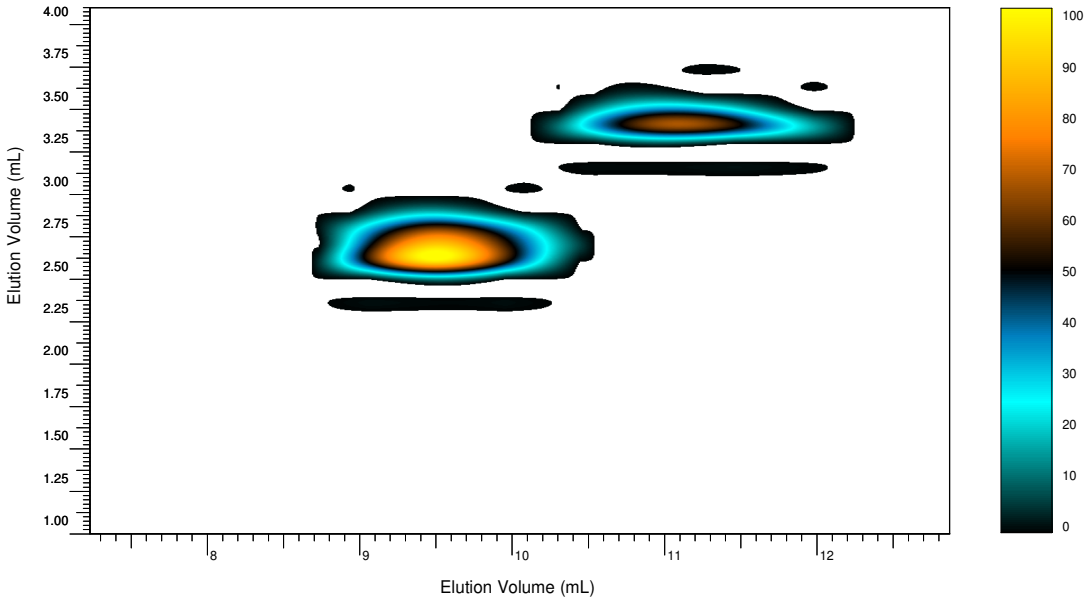

Figure 4.54: PS-b-PEO 6 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nd dimension: SEC with DMF as eluent.

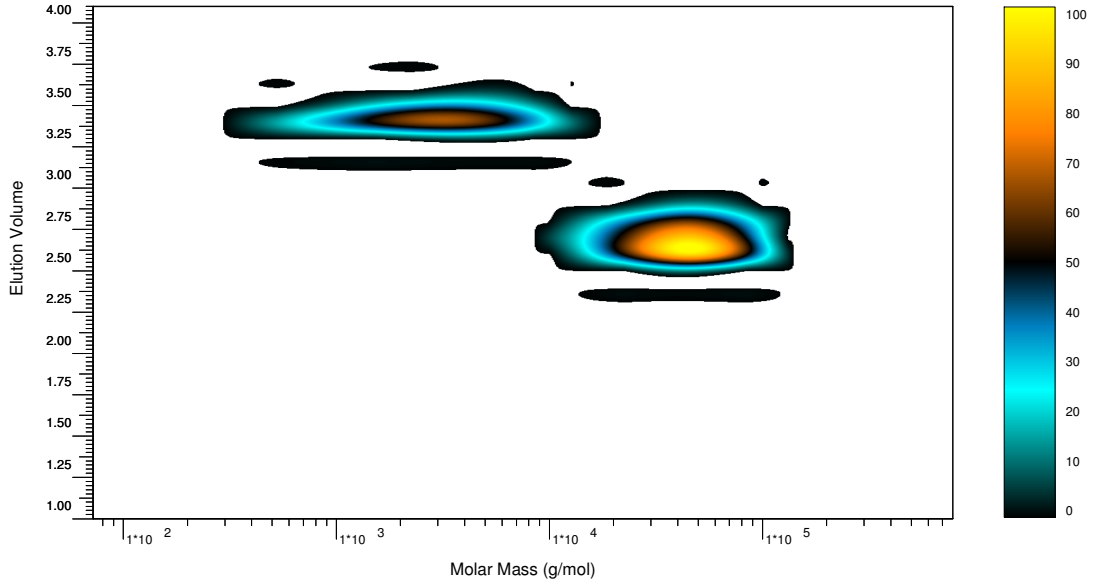

Figure 4.55: PS-b-PEO 6 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nd dimension: SEC with DMF as eluent. PEO calibration was applied.

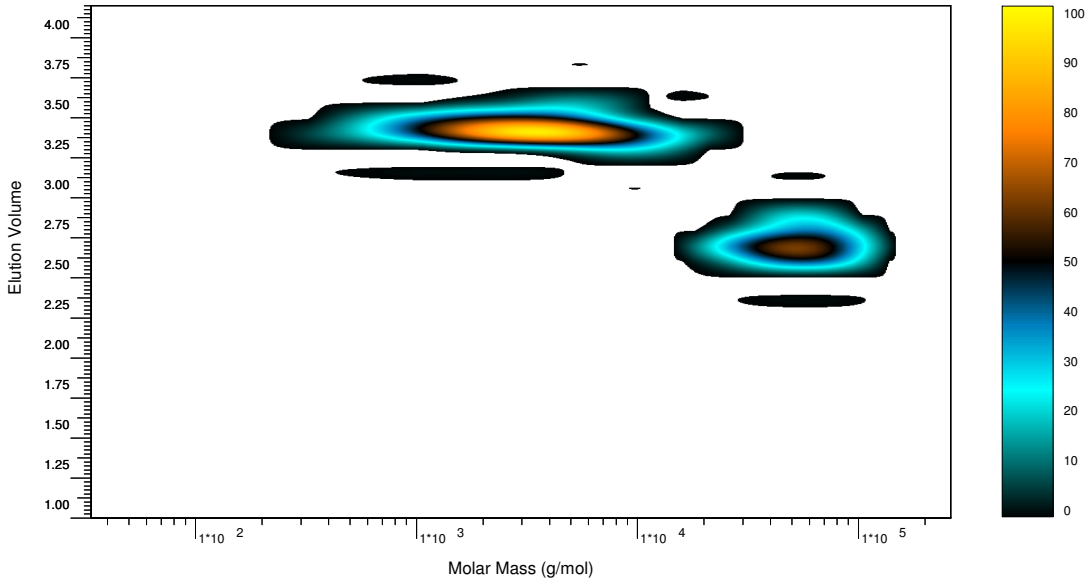

Figure 4.56: PS-b-PEO 7 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nddimension: SEC with DMF as eluent. PEO calibration was applied.

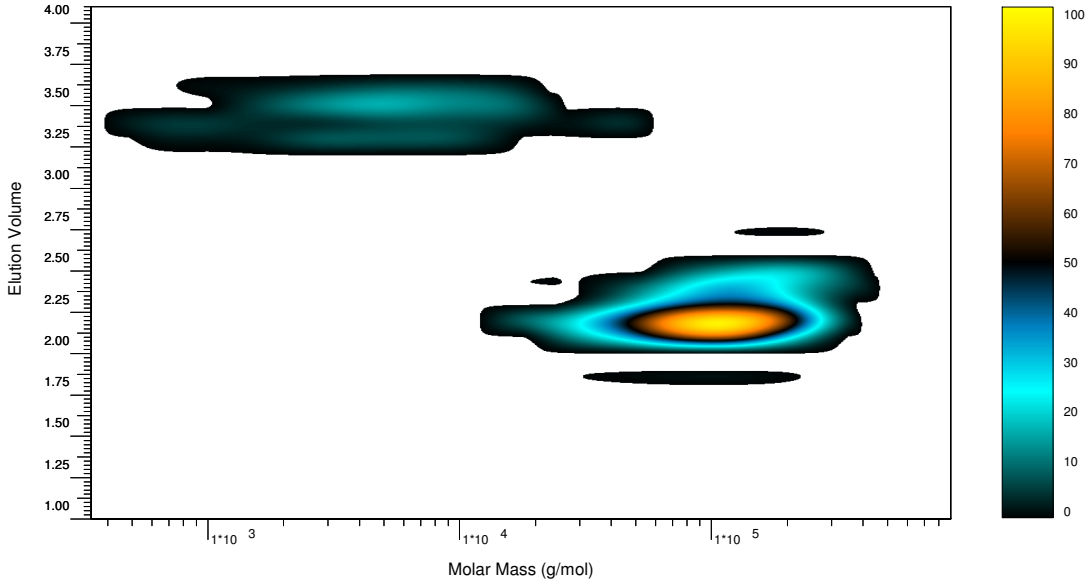

Figure 4.57: PS-b-PEO 8 2D-LC plot. 1st dimension: critical conditions of PEO (DMF:THF 4:96 vol.%) 2nddimension: SEC with DMF as eluent. PEO calibration was applied.

The molecular weight results for the PEO homopolymers as well as the copolymers obtained from the 2D plot for the eight samples are summaries in **Table 4.6**. These results were determined with the help of their corresponding V_e and the PEO calibration curve (**Figure 3.1**). When looking at the determined PEO homopolymer molecular weight results it

can be noted that they are much lower than what the manufacturer's data indicated. The molecular weight results for the block copolymer fractions (that might contain some PS homopolymer) are too low as compared to the manufacturer's data indicating that there is a fundamental problem with the PEO calibration. One possible explanation could be that the PEO calibration was done by injecting the calibration standards into the second dimension. These samples were dissolved in DMF. The real samples, however, are dissolved in DMF-THF when injected into the second dimension. The difference in the composition of the mobile phase might cause different hydrodynamic volumes and, hence, different calibration curves.

Table 4.6: Determined M_p for the PEO homopolymer and the copolymer fractions with the help of the 2D-LC (1^{st} dimension: critical conditions of PS (DMF:THF 4:96 vol.%) 2^{nd} dimension: SEC with DMF as eluent). PEO calibration curve was used.

	V_e of PEO homopolymer (mL)	M_p of PEO homopolymer (g/mol)	V_e of PS block copolymer & PS homo. (mL)	M_p of block copolymer & PS homo. (g/mol)
PS-b-PEO 1	-	-	11.42	2050
PS-b-PEO 2	-	-	11.29	2800
PS-b-PEO 3	-	-	10.88	5400
PS-b-PEO 4	10.53	8900	10.23	14200
PS-b-PEO 5	11.04	4200	10.08	19000
PS-b-PEO 6	11.36	2400	9.50	45500
PS-b-PEO 7	11.04	4200	9.40	50800
PS-b-PEO 8	11.00	4500	9.05	79900

For the quantification of the PEO homopolymer present in the original sample, a calibration curve for the ELSD was established as described in **Section 3.2.2 Figure 4.58** shows the calibration curves of PEO calibration standards with different molecular weights. It can be seen that the smallest peak area per injected mass is for M_p of 440 and 62000 g/mol of PEO calibration standards. For the very low molecular weight standard it can be assumed that partial evaporation takes place and only a fraction of the total sample is detected. For the higher molecular weight standards a clear trend is seen - the peak area decreases with

increasing molecular weight. This might be due to the fact that droplet formation changes with molecular weight or that some material is adsorbed on the stationary phase.

The amount of PEO homopolymer present in the copolymer samples can be calculated fairly accurately with the help of this calibration curve and the approximate M_p of the PEO homopolymer fractions obtained from the 2D-LC analysis.

Table 4.7 gives the percent content of PS and PEO homopolymer for PS-b-PEO 4 to PS-b-PEO 8 as well as their copolymer percentage. These results were obtained with the help of the two ELSD calibration curves for PS and PEO in **Figure 4.35** and **Figure 4.58** as well as the molecular weight results for the PS and PEO homopolymer from the 2D plots.

Table 4.7 was established with the help of the molecular weight data from the 2D plots for both critical conditions and with the two ELSD calibration curves (**Figure 4.35** and **Figure 4.58**). The amount of homopolymer for PS and PEO was determined with the two calibration curves and than subtracted from the total injected mass to obtain the amount of copolymer present in the original samples. The mass results were than converted to weight percentages. These results indicate that the samples 4-8 contain significant amounts of homopolymers while the samples 1-3 are more or less pure block copolymers.

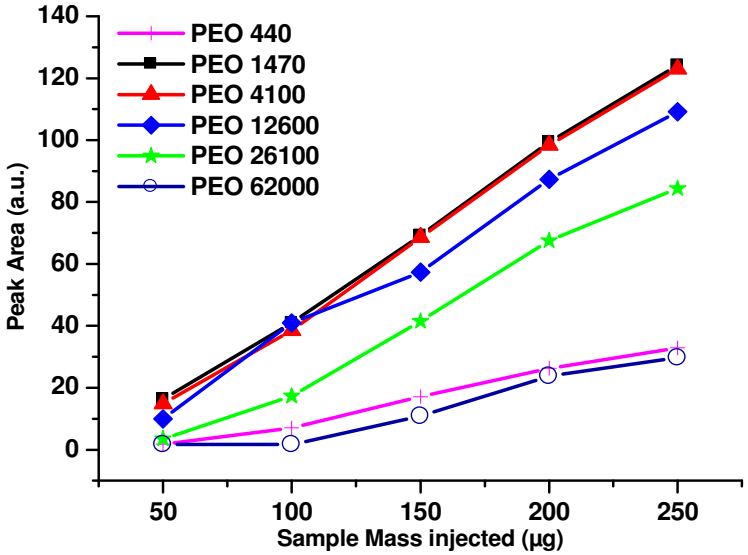

Figure 4.58: ELSD calibration curves for PEO with different molecular weights using 1D LCCC of PEO. ELSD conditions are 180°C for evaporation and 80°C for nebulisation at a N_2 gas flow rate of 1.5 SLM.

Table 4.7: Percent content of PS and PEO homopolymer and block copolymer present in the original samples.

	PS homopolymer (wt.%)	PEO homopolymer (wt.%)	Block copolymer (wt.%)
PS-b-PEO 4	0	58	42
PS-b-PEO 5	34	27	39
PS-b-PEO 6	0	48	52
PS-b-PEO 7	0	53	47
PS-b-PEO 8	33	27	40

4.5. References

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4. Baran, K.; Laugier, S.; Cramail, H. *Journal of Chromatography B: Biomedical Sciences and Applications* **2001,** 753, (1), 139-149.
5. Berek, D. *Chromatographia* **2003,** 57, 45-54.

Chapter 5

Conclusions and future work

5.1. Conclusions

For the critical conditions of PS, suitable solvents and solvent compositions were found which dissolve PS, PEO and PS-b-PEO. The samples and the calibration standards needed to be heated at approximately 40ºC to achieve good solubility. The critical conditions of PS were first established using a C-18 stationary phase with THF:H_2O as the solvent composition. It was found that this solvent composition system was not optimal, therefore THF:DMF was then used. The critical condition solvent composition was found to be at THF:DMF 82:18 vol.%. The block copolymers were analysed using the established critical conditions of PS but it was found, even though separation of PS homopolymer and copolymer was obtained, that PS blocks of the copolymers contributed to some extent to the retention of the PEO blocks and therefore the PEO block length could not be calculated. In order to obtain more insight into what the peaks are due to which were obtained from the separation at the critical conditions, some of the block copolymer samples were fractionated at that established critical conditions of PS. These fractions were qualitatively and quantitatively analysed using FTIR spectroscopy.

The settings for the 2D-LC analysis were established, using the critical conditions of PS as the first dimension and as the second dimension SEC using DMF as eluent. DMF was a suitable solvent to be used for the second dimension because PS, PEO and PS-b-PEO are soluble in it while in THF these samples were not completely soluble.

Similar procedure was followed to establish critical conditions of PEO. The same solvent combination as used for the critical conditions of PS could be used for the critical conditions of PEO but a different composition corresponded to the critical conditions of PEO. Critical conditions of PEO were established using a silica based stationary phase with DMF:THF as solvent composition. The critical condition solvent composition was found to be at DMF:THF 4:96 vol.%. The block copolymers were analysed using the established critical conditions of PEO but it was found, even though separation of PEO homopolymer and copolymer was obtained that PEO blocks of the copolymers contributed to some extent to the retention of the PS blocks and the PS block length could also not be determined. Some of the block copolymer

samples were fractionated at the established critical conditions of PEO. These fractions were qualitatively and quantitatively analysed using FTIR spectroscopy.
The settings for the 2D-LC analysis were established, using critical conditions of PEO as the first dimension and as the second dimension SEC using DMF as eluent.

Qualitative and quantitative analyses of the block copolymers were carried out using FTIR spectroscopy. The quantitative FTIR analysis was quite a challenge due to the reason that DMF was used as the solvent for the samples. The DMF bands absorbed in the same frequency region where the PEO bands are absorbing, therefore it was not easy to find an appropriate PEO band which could be used for the quantification.

5.2. Future work

- To investigate the reason why at, for example, the LCCC of PS the lower molecular weight PEO calibration standards elute at such a low elution volume compared to the elution volume of the low molecular weight PS calibration standard. The same needs also to be investigated when working at LCCC of PEO and why the lower molecular weight PS calibration standards elute so much earlier than the low molecular weight PEO calibration standards. A possible approach would be to use some volatile salt such as ammonium acetate or ammonium trifluoroacetate, but first it needs to be tested if these salts will dissolve in the solvent compositions which were used for the LCCC.
- To investigate the reason why the critical polymer part contributes to the retention of the non-critical polymer part. A possibility would be to find another (new) solvent composition system, which should be able to dissolve the block copolymers and the two types of calibration standards (which is not an easy task), and to see if the contribution problem is solved. Then it might be possible to determine a more accurate block length of the non-critical copolymer blocks.
- When having found another solvent (one of the two solvents used for the solvent composition system) which dissolves the block copolymers and the two types of calibration standards to repeat the quantitative FTIR analysis since it was difficult to

find an appropriate band which could be used for the quantification of PEO when using DMF as solvent.